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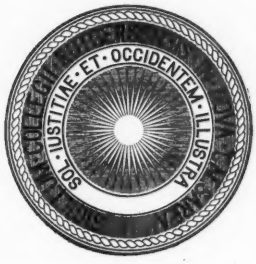

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SOIL SCIENCE

Contents for March, 1922

J. R. NELLER. The Influence of Growing Plants upon Oxidation Processes in the Soil..... 139
JACOB S. JOFFE. Preliminary Studies on the Isolation of Sulfur-Oxidizing Bacteria from Sulfur-Floats-Soil Composts..... 161
A. F. GUSTAFSON. The Effect of Drying Soils on the Water-Soluble Contsituents..... 173
W. RUDOLFS. Sulfur Oxidation in "Black Alkali" Soils..... 215

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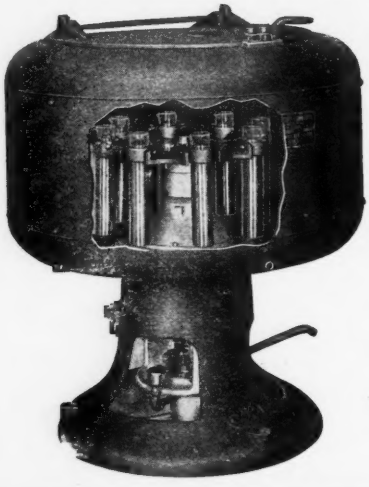

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THE INFLUENCE OF GROWING PLANTS UPON OXIDATION PROCESSES IN THE SOIL[1]

J. R. NELLER

New Jersey Agricultural Experiment Stations

Received for publication June 23, 1921

INTRODUCTION

That a symbiotic relationship exists between leguminous plants and specific strains of nitrogen-fixing bacteria was finally established only after decades of investigation. The knowledge and control of this relationship has been of great value to agriculture and it is probable, that equally important relationships exist between other groups of soil microörganisms and the growing roots of cultivated plants. Considering the physical and chemical activities involved in the growth of roots and the biochemical activity of the soil organisms in a productive soil, it appears impossible that the groups or sets of reactions involved in the life processes of such widely differing organisms could mingle so intimately and not have a marked effect upon each other. Certain types of microörganisms may function more actively upon the surfaces or in the immediate vicinity of the rootlets and root hairs of green plants than upon the particles of the soil complex not in contact with the growing roots. There is reason to believe that the oxidizing flora of the soil is thus stimulated by contact with, or by close proximity to the growing roots of the higher plants.

Carbon dioxide which is generally taken as the index of biochemical oxidation is, of course, liberated into the soil by the roots of growing green plants as well as by the organisms of decay. Therefore measurements of the carbon dioxide found in the soil atmosphere of planted and unplanted areas can be of no aid in determining whether growing plants retard or hasten the liberation of plant nutrient material as it is affected by the oxidation processes.

The experiments reported in the following pages were planned and executed for the purpose of ascertaining whether growing green plants have any influence upon the rate of oxidation of soil organic matter.

REVIEW OF LITERATURE

The literature cited bears upon the factors which cause and influence oxidation processes in the soil including the effects which growing plants have upon each other as well as upon soil biological activities.

[1] Paper No. 44 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Plant Physiology.

About the middle of the last century Boussingault and Lewy (5) observed that the decomposition of soil organic matter required more oxygen than was present in the organic matter itself. Later Schloessing (52) found that the soil atmosphere contained less oxygen and more carbon dioxide than the atmosphere above the soil. Wollny (65) carried out some extensive experiments on oxidation by measuring the amount of carbon dioxide set free by aerating a given amount of soil. He was the first to attribute the oxidation to microorganisms.

More recently Russell (48) has reported work in which he measured the amount of oxygen absorbed or utilized by soil organisms. He considered that most of the oxidizing agents were aerobic and that the oxygen absorption method gave a better index of biological activities than the method of Wollny, because considerable of the carbon dioxide liberated is adsorbed and retained by the soil.

Knowing that living cells are concerned in the process, it was but natural to expect that enzymatic action plays a part. The presence of active oxidase and peroxidase in soil has been reported by Woods (66) and Koenig (27), while Schreiner and Reed (53) have shown that growing roots can cause considerable oxidation which they attribute to the extracellular action of oxidase. Schreiner and Skinner (54) have pointed out that the soil oxidation activities not only liberate large quantities of plant nutrient material but that they are also necessary for converting toxic substances into harmless and beneficial compounds.

Several investigators have studied the influence which various materials have upon the oxidizing power of the soil. Lemmerman and his associates (32) report the influence of calcium carbonate. Lipman and Blair (35) ascertained the effect of adding nitrates and carbohydrates to the soil while Fred and Hart (14) compared the effects of sulfates and phosphates. Others who have included carbon-dioxide determinations in their soil researches are Potter and Snyder (46, 47), Van Suchtelen (62), Gainy (15) and Neller (43, 45).

For many years it has been observed that in a mixed growth of legumes and non-legumes the latter appear to grow better than they do when planted alone. After it became known that the growth of legumes increased the nitrogen content of the soil it was suggested that the non-legumes were able to utilize some of the nitrogen during the same season that it was being fixed in the root tubercles of the legumes.

By means of a system of porous and non-porous cups Lipman (33) demonstrated that growing peas exerted a beneficial influence upon the growth of oats. Further investigations upon the nitrogen content of the plants as well as upon the effect of adding sodium nitrate made it apparent that nitrogenous compounds passed out of the roots of at least some legumes and became available to non-legumes associated with them.

Lyon and Bizzell (36) report field experiments in which it was found that timothy grown with alfalfa or red clover contained a greater percentage of

nitrogen than timothy grown alone. Since the rate of nitrification of ammonium sulfate was greater in the alfalfa soil, they suggested that the favorable effect upon the non-legume was due to a more rapid formation of nitrates where legumes were growing. Westgate and Oakley (64) consider that their results conflict with those of Lyon and Bizzell since they found that an increasing amount of wheat planted with clover decreased the protein content of both wheat and clover.

Kellerman and Wright (23) did not find any increase in the nitrifying power of a semi-arid soil previously planted with legumes. They point out that the effect of a crop upon the nitrifying power may be very different in different soils. It seems evident, however, that non-legumes generally benefit from an association with legumes provided the soil and climatic conditions are normal.

In England the peculiar and decidedly toxic effect of grass grown in conjunction with fruit trees has been quite conclusively demonstrated by Bedford and Pickering (2) who consider that the toxin results from the dead organic matter of the grass roots and that it is rendered harmless by oxidation. This is in accord with previous work by Schreiner and Skinner (54) who found that the harmful influence of a soil extract may be removed by processes which promote oxidation.

The effect of plant life upon the oxidizing power of the soil has been given less attention than its effect upon the nitrifying power. This, probably has been due to the fact that the question of nitrates has been a pressing one and also because it is difficult to measure the rate or determine the nature of oxidizing reactions. Schreiner and Sullivan (55) found that the potential oxidizing power of the soil determined by shaking its water extract with a solution of aloin was increased by 75 per cent as a result of 17 days' growth of wheat. The effect upon a poor, sandy soil, however, was not so pronounced as it was upon one which was manured and more productive.

In some recent studies of the soil atmosphere, Russell and Appleyard (51) concluded that the effect of a growing crop was to increase the carbon dioxide content of the soil atmosphere. They are in doubt as to whether or not the growing crop might be credited with any stimulating effect upon bacterial activity, but do consider that there is not much evidence of a depressing effect such as Russell mentioned previously (49). Turpin (61) states that the carbon-dioxide production in a cropped soil was markedly greater than that in a soil in which no crop was growing. It was concluded that the excess carbon dioxide in a cropped soil is due to the respiratory activity of the plants rather than to the decay of the root particles from the crop growing on the soil at the time of the analysis.

Turning now to the question of the effect of crops upon nitrate production, it may be found that although a considerable number of investigators have studied the subject they differ widely in their conclusions. Lawes, Gilbert, and Warington (29) were among the first to record this apparent influence of

crops. They state that the nitrogen of unmanured land nitrifies with more difficulty than the nitrogen of land that has yielded large crops.

Later King and Whitson (24) found that the nitrate used by crops (tops only) indicated either a greater nitrification of the original soil organic matter where legumes were growing or that the legumes utilized considerable nitrogen directly from the air during the growth of the plant. The following year they reported a more definite experiment (25). The nitrogen in a crop of oats and the soil nitrate to a depth of 4 feet were determined on June 20. One-half of the oats were then harvested. Nineteen days later the nitrates in both halves of the plot were determined. It was found that the nitrogen utilized by the crop during the 18-day interval plus the nitrogen of the soil nitrates was about twice as great as the nitrogen of the accumulated nitrate on the bare portion of the plot. Had the nitrogen of the roots been considered, the apparent increased nitrification in the cropped soil might have been still greater. The authors could give no explanation for the marked increase. Fraps (13) reports a somewhat similar result with maize grown in a greenhouse, except that the soil was uncropped from the beginning of the growing season. However, great variations in the moisture and aeration conditions occurring in his tests might have caused the increase in nitrate production in the cropped soil.

Lyon and Bizzell (37) found more nitrates in land upon which maize was grown than in a similar unplanted soil. But during the later periods of growth there was less nitrate accumulation than in the cropped soil. They advance the theory that the growing maze stimulates the formation of nitrates at first and later inhibits it when the roots cease to grow and begin to decay. These authors found (39) that even after an alfalfa soil had been kept fallow for 2 years, it nitrified dried blood more rapidly than did a timothy soil similarly treated. They also report some recent work (40) in which six lysimeters were planted with clover and six with timothy. Oats and maize were planted the following year and the total production of nitrates was determined for each lysimeter. Those which contained the clover soil caused the greater production of nitrates in spite of the fact that ample quantities of dried blood were added to each soil. However, the increased nitrate production in the clover soil may have been due to the fact that the clover roots were more easily nitrified than the dried blood.

It is interesting to note that several investigators have found that the nitrate production under a cultivated crop such as corn or potatoes is higher than under an uncultivated crop such as wheat, oats, or timothy. Such were the findings of Jensen (21) in South Dakota, Brown and MacIntire (7) in Pennsylvania, Ladd (28) in North Dakota, and Lyon and Bizzell (38) in New York. These results appear to obtain chiefly in prairie soils which are generally well supplied with humus. Here more frequent cultivation would probably cause a more intensive nitrification.

The semi-arid soils of the United States present a set of conditions much different from those of the humid regions, and Stewart and Greaves (58) report that the nitric nitrogen in corn and potato land and in fallow land was high in the spring and summer but comparatively low in the fall, while the nitric nitrogen of alfalfa land was low throughout the year. A later report from Utah (17) concludes that alfalfa not only feeds more closely upon the nitric nitrogen of the soil than do other crops, but that it also increases the nitrifying power of the soil. McBeth and Smith (41) were led to conclude that the nitrifying power of a cropped and irrigated soil was higher than that of one which was not cropped.

As opposed to the conclusion that crops stimulate nitrification in the soil, a number of investigators have obtained experimental evidence that growing crops have no influence upon nitrification or produce a retarding effect upon this process. Deherain (11) concluded that if moisture conditions were always the same and if no loss occurred through drainage the nitrate production would be the same in both fallowed and cropped soils. Vorhees, Lipman, and Brown (63) found that nitrate accumulation was slower under oats and clover than it was in fallow soils. Taking into account the nitrate nitrogen used by the crops and that remaining in the soil Russell (49) concluded that less nitrate nitrogen was produced in cropped than in uncropped soils. However, in his later studies (51) of the soil atmosphere he found no evidence of any depressing effect of crops upon nitrate production.

In India, Leather (30) determined the nitrate nitrogen in a soil from which the previous accumulation of nitrates had been leached by a heavy rainfall and found that less nitrate was produced in this soil where grass was growing upon it than where no vegetation occurred. Kellerman and Allen (22) state that in their irrigated soils the age and condition of an alfalfa field bore little if any relation to the potential rate of nitrification.

Berthelot (3) was probably one of the first to observe the effects of growing crops upon bacterial activities other than those of the nitrifiers. He states that there was less rapid fixation by free living organisms in cropped than in uncropped soils but considers that this may have been due to the fact that the plants used some of the fixed nitrogen. On the other hand, Heinze (19) found that the practice of fallowing increased the nitrifiers, ammonifiers and azofiers.

Caron (8) reports that a greater number of organisms were present under clover than under grain crops. Stoklasa and Ernst (59) counted from seven to eight millions of colonies per gram of clover soil, while comparable figures gave five to six millions in barley soil and one to two millions in soil growing sugar beets. Leclair (31) also found higher bacterial numbers under cowpeas than in soil of fallow plots. An increase in bacterial numbers and in the nitrifying and ammonifying power of soil under rotation cropping as opposed to continuous single-cropping systems are the results submitted by Brown (6).

Thus the evidence appears to show that growing crops do not exert a depressing effect upon biological activities in soil. Neither does it seem definitely proven that they exert a stimulating effect, and to clear up the matter there is need of comprehensive experiments under conditions of better control than those which have so far been reported. Russell (50) realized such a need when he recently stated, "there appears to remain only the possibility that the growing crop has a direct influence upon the decomposition processes going on in the soil. Unfortunately field experiments alone do not enable us to decide this question and the systematic laboratory investigation has still to be undertaken."

PRINCIPLES INVOLVED IN THE EXPERIMENTAL WORK

These experiments were planned for the purpose of ascertaining the influence of growing roots upon oxidation activities that take place in soil. This was done by comparing two lots of the same sample of soil, the one planted and the other unplanted, with all other conditions such as moisture, aeration, and supply of plant nutrients as nearly as possible the same for each soil.

The amount of carbon dioxide evolved from a soil is an index of oxidation activities occurring therein. Under normal conditions, of course, a part of the carbon dioxide produced in a soil is due to the respiration of growing plants and a part to the activities of microörganisms. The conditions of the experiments required that the latter source of production be accurately measured. This was accomplished by placing the soil and growing plants in enclosed systems through which air, freed from carbon dioxide, was drawn. Thus the only source of carbon dioxide was that set free by the decomposition processes taking place in the soil contained within the systems. The carbon dioxide thus utilized by the growing plants in the photosynthetic processes and that respired, was first produced by the microörganisms of the soil in which the plants were growing.

The total carbon dioxide evolved in the enclosed systems without plants was determined by means of passing a slow but continuous current of carbon-dioxide-free air into the systems and then out again through absorption towers in which the carbon dioxide was retained and could be measured. In the systems containing growing plants, some of the carbon dioxide evolved from the soil was, of course, fixed by the plants in the process of photosynthesis and retained. This fixed carbon dioxide was determined by making a total carbon analysis of the plants. Thus the total amount of carbon dioxide produced by microörganisms in planted and unplanted soils was measured quantitatively.

DESCRIPTION OF APPARATUS

The apparatus with which most of the work was done consisted of twelve separate units, each of which employed a glazed earthenware jar *A* (fig. 1) to which was cemented a square board *B* by means of shellac. This served as a base for an inverted battery jar *C* which was

22 cm. in diameter and 30 cm. high. The center of the board was removed to correspond to the inner diameter of the glazed jar and was grooved on its upper surface to receive the lower edge of the inverted battery jar. After having been sealed to the lower jar the wooden base was thoroughly impregnated and coated with hot paraffin wax.

The air which was drawn into the apparatus was freed from carbon dioxide by passing it through a tube 22 mm. in diameter and 330 cm. long, lying in a horizontal position and loosely filled with 4-mesh soda lime (made up in a moist condition for carbon-dioxide determinations). From thence it was bubbled through a 10 per cent sulfuric acid solution contained in each of the twelve distributing bottles *D*. The acid solutions in the distributing bottles

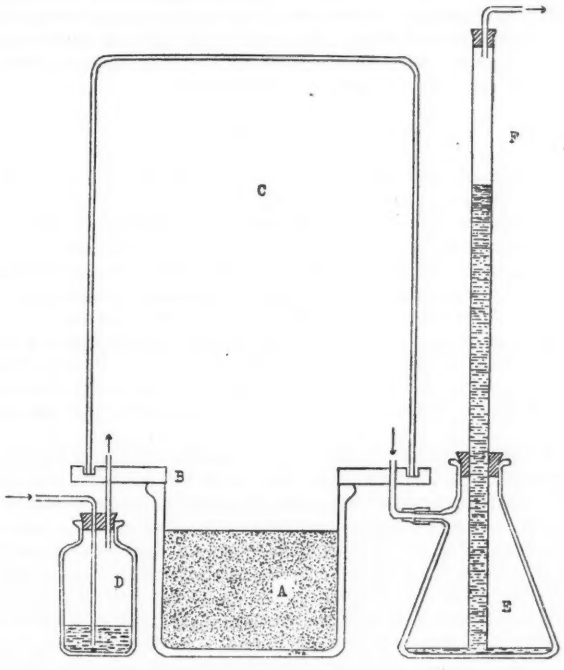

FIG. 1. DIAGRAM REPRESENTING A SECTION THROUGH ONE OF THE UNITS OF THE APPARATUS USED IN DETERMINING THE CARBON DIOXIDE EVOLVED FROM THE SOIL CULTURES

prevented diffusion of carbon dioxide from one unit to another. The outdrawn air from each unit was led through a modified Truog (60) absorption tower *E*. Slow but continuous aspiration was maintained by means of a suction pump which was supplied with water from a constant level tank. Figure 1 of plate 1 shows the twelve units as seen from the front or outlet side. Figure 2, the rear view, shows how the indrawn air was distributed to each unit.

The tendency to create a partial vacuum within the apparatus was very slight, being approximately equivalent to the 3-cm. columns of solution contained in the distributing bottles. The main difficulty in the way of keeping each system air-tight was in the unequal expansion and contraction of the apparatus caused by the fluctuating temperature of the greenhouse. This unequal expansion and contraction was especially marked between the inverted battery

jar *C* (fig. 1) and the wooden base *B*. Molten paraffin was first used to fill the circular groove and thus seal the jar to its base. This allowed leaks to occur after a time because the expanding and contracting glass caused the paraffin to be pushed away from its inner wall. Vaseline failed to prevent leakage for much the same reason.

Neither sealing wax nor shellac could be used because it was necessary to remove the jars from time to time. A wax was needed which would adhere to the glass and be elastic enough to withstand the expansion and contraction without rupturing. To meet these requirements various mixtures of rubber, beeswax, paraffin and vaseline were tested. The wax finally used was made by melting down old rubber stoppers with a very small amount of paraffin. A small amount of sulfur was added and the heating continued under a hood until the mixture had the desired melting point. It was found that the consistency and the melting point limits were determined largely by the amount of heating and so a definite formula for the mixture can not be given. With the use of this wax the apparatus was kept practically free from leakage, but not without constant care and attention.

EXPERIMENTAL PROCEDURE

The sand and the soil which was used as a culture medium was put through a 3-mm. sieve and weighed out into 2000-gm. lots, one for each of the jars of the apparatus. When pure sand or a soil deficient in humus was employed it was thoroughly mixed with a definite amount of organic matter. This consisted of a humus preparation made by mixing finely ground alfalfa hay with soil diluted with sand. This mixture was moistened and occasionally stirred for about two weeks, by which time the more easily decomposable portions had become oxidized. It was then dried at 100°C. and stored for future use.

Seedlings were germinated in paraffined drinking cups containing some of the same lot of soil which was used in the jars to which they were transplanted. By this method all of the soil in the cups could be transferred to the apparatus without greatly disturbing the roots of the growing plants. Cups of similarly treated unplanted soil were transferred to the jars used as checks.

As soon as the seedlings were transplanted, the required amount of water or of nutrient solution was added to the solid substratum and the jars were sealed. A slow but continuous current of air was then started through the systems and the carbon dioxide which was not fixed by the plants in the photo-synthetic processes was absorbed in the barium hydroxide towers. The total carbon dioxide absorbed in these was determined quantitatively after shaking the glass beads out of the tube *F* (fig. 1) into the flask *E* containing the residual barium hydroxide, rinsing the tube with carbon-dioxide-free water and then titrating the residual barium hydroxide with oxalic acid, phenolphthalein being used as the indicator. A complete refilling of the towers was necessary every 1 to 3 days. On several occasions the barium hydroxide in some of the towers became neutralized during the night hours, thus involving the possibility of a slight loss of carbon dioxide. But since special precautions were taken to avoid such occurrences it is considered that the error thus occasioned was negligible.

Since the plants were grown in an enclosed space the atmosphere surrounding them was approximately saturated with moisture during the greater part of the growth period. This, of course, checked transpiration and affected the growth of the plants in much the same way as that described by Eberhart (12). The stems were longer and smaller in diameter and the leaves were somewhat smaller than those of plants grown in drier air. Newcomb and Boverman (42) working with seedlings in the dark found that growth in unventilated chambers was not retarded. In the present work good growth was obtained and the plants appeared vigorous and healthy except in one or two instances when some of the plants were injured by too high temperatures in the summer months.

The carbon-dioxide content of the air surrounding the plants was considerably higher than that of atmospheric air at the beginning of the experiments, but was probably lower at their termination about 30 days later. It has been reported by Boussingault (4) and later

by Cummings and Jones (10) that the most rapid plant growth takes place in an atmosphere containing about 8 per cent of carbon dioxide.

When it was desired to terminate an experiment, air was drawn through the systems rapidly for two or three hours in order to remove the carbon dioxide as completely as possible. The plants were then removed and analyzed for carbon. It was impossible to separate all of the finer rootlets from the soil. A slight growth of algae was observed on the surface of the soil in the jars, but as this occurred on both the planted and unplanted soil it is not probable that the comparative results were appreciably altered. In some cases weed growth developed in the unplanted jars, in which event they were harvested and the carbon dioxide, as represented by their total carbon content, was added to the amount obtained in the absorption towers for those jars.

All the plants from each culture were dried to constant weight at 104°C., weighed and ashed. The loss by ignition minus that of an equal number of seedlings of approximately the same size as those planted, was taken to represent the amount of dry plant material produced during the period of the experiment. In order to reduce any possible error the seedling blank was obtained by burning from three to four times the number of seedlings which were planted in one jar and calculating for the required number. The carbon determinations were made according to the official method of the Association of Agricultural Chemists (1).

It is known, of course, that the ash of plants contains carbonates the carbon of which should be included as part of the total carbon of the plant. This was not done in the present experiments. However, if this carbon of the ash had been considered in the present work, the results obtained would have been even more positive. Therefore, it cannot be considered that the error thus introduced could detract from the results obtained.

INFLUENCE OF GREEN PLANTS UPON THE OXIDATION OF ORGANIC MATTER MIXED WITH SAND

A washed white sand having a maximum water-holding capacity of 30 per cent by the Hilgard method (20) was inoculated by mixing it with 10 per cent of its weight of a fertile loam soil. Two thousand grams were used per jar with which 3 gm. of finely ground soybean hay were thoroughly mixed. Six soybean seedlings, 10 days old, were planted in each of the jars numbered 11 and 12 (table 1) and the soil in all of the jars was then moistened to 50 per cent of its water-holding capacity with a nutrient solution. The inverted battery jars were then sealed down and aspiration of air free from carbon dioxide started through them on November 12, and continued for the next 29 days. By that time many of the upper leaves were pressing against the tops of the enclosing jars.

The data of table 1 clearly show that the total carbon dioxide recovered from each of two planted jars was considerably more than the total amount obtained from the corresponding unplanted jars, the average for the latter being 1737.0 mgm. and for the former 2093.4 mgm. The average total carbon dioxide evolved from the planted jars is thus 12.1 per cent higher than the average total yield from the unplanted jars. It appears, therefore, that the growing plants exert a pronounced accelerating influence upon the oxidation activities taking place in the sand cultures. It is interesting to note in this connection that in some preliminary work (43) previously carried out in a similar manner, the influence of plants growing in sand cultures was even more pronounced

than that here shown, the average total amount of carbon dioxide recovered from jars planted with wheat and from those planted with barley being 25.2 per cent and 12.9 per cent higher, respectively, than the corresponding amount recovered from the unplanted jars.

TABLE 1

Effect of soybeans on the oxidation processes in sand to which equal amounts of soil and organic matter were added. Experiment conducted from November 12 to December 10, 1919

	INTERVALS BETWEEN TITRATIONS	CHECK (NO PLANTS)		SOYBEANS	
		Jar 9	Jar 10	Jar 11	Jar 12
	days	*mgm.*	*mgm.*	*mgm.*	*mgm.*
CO_2 withdrawn from system	0–3	252.6	227.0	253.4	288.0
	4–5	165.5	167.0	167.0	167.0
	6–7	167.0	167.0	101.4	167.0
	8–9	161.9	159.5	129.0	167.0
	10–11	170.1	244.9	260.9	154.9
	12–14	196.5	221.4	76.2	54.9
	15–16	147.6	150.0	69.5	150.0
	17–21	165.0	178.8	15.0	69.2
	22–26	132.9	153.4	14.0	23.7
	27–29	149.6	96.3	48.4	47.2
Total		1708.7	1765.3	1134.8	1288.9
Dry weight of crop				1278.7	1188.5
Ash in crop				239.3	246.9
Organic matter in crop				939.4	941.6
Organic matter in seedlings				430.0	430.0
Organic matter produced during experiment				509.4	511.6
CO_2 fixed by plants during experiment				879.7	883.5
Total CO_2 obtained from soil		1708.7	1765.7	2014.5	2172.4
Average CO_2 obtained from soil		1737.0 mgm.		2093.4 mgm.	
Average increase over check				12.1 per cent	

TABLE 2

Moisture content of cropped and uncropped soils kept under enclosed systems

	EXPERIMENT 1 NOVEMBER 2 TO DECEMBER 10				EXPERIMENT 3 NOVEMBER 27 TO DECEMBER 27			
	Uncropped jars		Cropped jars		Uncropped jars		Cropped jars	
	1	2	1	2	1	2	1	2
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Initial moisture content	15.0	15.0	15.0	15.0	24.0	24.0	24.0	24.0
Final moisture content	13.6	14.2	11.2	12.4	22.3	20.3	22.4	21.3

The apparatus by means of which the first experiment was conducted was equipped with calcium chloride tubes to permit a measurement of the amount of water lost from the enclosed jars of sand. It was found by weighing the

tubes as well as by making moisture determinations of the sand at the close of the experiment that only a slight loss of moisture occurred. This is indicated in table 2 which shows also that when soil was used there was no appreciable loss of moisture. It may be concluded, therefore, that differences observed in the total carbon dioxide evolved from the planted and unplanted soils were not caused by variations in their moisture contents.

INFLUENCE OF GREEN PLANTS UPON THE OXIDATION OF ORGANIC MATTER IN A LOAM SOIL

No additional organic matter was added to the soil used in this experiment. It contained 1.218 per cent of organic carbon and had a maximum water-hold-

TABLE 3

Effect of buckwheat and of field peas upon the oxidation processes in a loam soil. Experiment conducted from November 29 to Decembe 30, 1919

	INTERVALS BETWEEN TITRATIONS	BUCKWHEAT		CHECK (NO PLANTS)		FIELD PEAS	
		Jar 1	Jar 2	Jar 3	Jar 4	Jar 5	Jar 6
	days	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
CO_2 withdrawn from system	0–3	88.9	85.0	81.7	74.6	112.3	112.3
	4–5	23.9	15.8	46.5	34.2	55.1	47.6
	6–7	76.7	61.1	33.0	38.9	25.1	38.0
	8–10	39.7	6.5	32.0	21.7	37.3	33.7
	11–13	49.0	21.8	58.3	58.1	55.0	36.8
	14–18	79.6	40.0	41.4	46.8	7.9	10.5
	19–23	44.4	46.0	69.3	43.0	22.0	62.1
	24–32	27.0	20.1	121.0	79.7	22.1	15.8
Total		429.2	296.3	483.2	397.0	336.8	356.8
Dry weight of crop		786.8	842.8			1032.5	1024.6
Ash in crop		347.0	367.0			317.9	405.2
Organic matter in crop		439.8	475.8			714.6	619.4
Organic matter in seedlings		92.7	92.7			416.7	416.7
Organic matter produced during experiment		347.1	383.1			297.9	202.7
CO_2 fixed by plants during experiment		561.3	619.5			418.7	327.8
Total CO_2 obtained from soil		990.5	915.5	483.2	397.0	818.5	684.6
Average CO_2 obtained from soil		953.1 mgm.		440.1 mgm.		751.6 mgm.	
Average increase over check		116.5%				70.8%	

ing capacity of 37.5 per cent. It was nearly neutral in reaction as the pH value of its water extract was 6.7 determined colorimetrically according to the method of Gillespie (16) and with the indicators described by Clark and Lubs (9). When ready for use, the soil was brought to one-half of its maximum water-holding capacity by the addition of Shive's (56) solution no. R5C2 at 1.75 atmospheres concentration. Buckwheat and field pea seedlings were

transplanted, six plants to each jar, on November 29 and harvested on December 30.

The recovery of carbon dioxide is given in table 3. Jars in which buckwheat was growing showed an increase of 116.5 per cent over the amount obtained from the unplanted jars; field peas produced a corresponding increase of 70.8 per cent.

A few months later some of the same lot of soil was tested in a similar manner except that soybeans were used instead of field peas and buckwheat. In this test, also, the growing plants exerted a stimulating influence upon the

TABLE 4

Effect of soybeans on the oxidation processes in a loam soil. Experiment conducted from May 24 to June 16, 1920

	INTERVALS BETWEEN TITRATIONS	SOYBEANS			CHECK (NO PLANTS)		
		Jar 1	Jar 2	Jar 3	Jar 4	Jar 5	Jar 6
	days	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
CO_2 removed from system	0–6	206.2	136.2	235.2	399.4	394.9	340.2
	7–10	149.0	106.8	207.0	225.1	199.8	209.8
	11–14	137.5	77.0	165.3	210.0	93.7	156.9
	15–17	144.9	123.2	199.2	228.8	228.8	122.8
	18–24	280.5	175.1	232.2	455.8	263.4	328.2
Total		918.1	618.3	1038.9	1513.1	1174.6	1157.9
Dry weight of crop		1402.0	1764.8	1547.8			
Weight of ash		227.8	625.5	320.3			
Organic matter in crop		1124.2	1139.3	1227.5			
Organic matter in seedlings		425.0	425.0	425.0			
Organic matter produced during experiment		699.2	714.3	802.5			
CO_2 fixed by plants during experiment		1201.9	1227.9	1379.5			
Total CO_2 obtained from soil		2120.0	1856.2	2418.4	1513.1	1174.6	1157.9
Average CO_2 obtained from soil		2128.2 mgm.			1281.8 mgm.		
Average increase over check		66.0%					

oxidation activities of the soil. Table 4 shows that the carbon dioxide evolution, averaged from triplicate determinations, was 66.0 per cent higher than the corresponding amount obtained from the unplanted soil.

THE INFLUENCE OF RECROPPING UPON OXIDATION PROCESSES IN THE SOIL

In this experiment the same jars of sand which were used in the first experiment were replanted with wheat and soybeans, the check soils always remaining unplanted. A comparison of table 1 with table 5 shows that the second crop of soybeans appeared to have much greater accelerating influence than the first crop upon the oxidation activities taking place in the sand medium

in which the plants were grown. Table 5 shows the average total carbon dioxide recovered from the cultures planted with wheat and from those planted with soybeans to be 100.9 per cent and 108.8 per cent higher, respectively, than the average amount recovered from the check cultures.

An augmented effect of the second crop upon oxidation activities was also obtained with the loam soil previously used. The total carbon dioxide re-

TABLE 5

Effect of the second crop of wheat and of soybeans upon the oxidation processes in sand cultures. Experiment conducted from December 18 to January 10, 1920

	INTERVALS BETWEEN TITRATIONS	WHEAT		CHECK (NO PLANTS)		SOYBEANS	
		Jar 7	Jar 8	Jar 9	Jar 10	Jar 11	Jar 12
	days	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
CO_2 removed from system	0–3	191.1	191.1	191.1	181.1	191.1	182.1
	4–6	187.0	187.0	175.5	187.0	187.0	187.0
	7–9	108.9	170.0	178.0	97.1	89.4	167.0
	10–12	129.7	143.4	83.0	109.2	135.2	94.2
	13–14	40.6	43.8	68.1	51.0	65.5	55.0
	15	84.7	99.4	165.1	107.0	95.9	123.0
	16–19	48.0	75.0	108.1	116.0		24.9
	20–24	21.8	50.5	326.3	349.0	36.8	43.4
Total		811.8	960.4	1295.3	1098.3	780.9	876.6
Dry weight of crop		1280.8	2756.5			1442.5	1523.6
Ash in crop*		220.0	1916.0			394.0	424.2
Organic matter in crop		1080.8	840.5			1048.5	1099.4
CO_2 used to produce organic matter in crop		1747.4	1359.1			1695.4	1783.9
CO_2 used to produce organic matter of seedlings		26.9	26.9			69.5	69.5
CO_2 fixed by plants during experiment		1702.8	1332.2			1625.9	1714.4
Total CO_2 obtained from soil		2514.4	2292.6	1295.3	1098.3	2406.8	2591.0
Average CO_2 obtained from soil		2403.6 mgm.		1196.8 mgm.		2498.9 mgm.	
Average increase over check		100.9%				108.8%	

* Including also the soil grains which adhered to roots.

covered from this soil when replanted with soybeans was 106.6 per cent greater than that obtained from the unplanted jars, as is indicated in table 6. For the first planting the corresponding increase was only 66 per cent. In this connection it should be noted that the soil which was used to support the first crop was not allowed to become dry before the second crop was planted. This immediate replanting may have had something to do with the increased percentage of carbon dioxide recovery over that recovered during the growth of the first crop.

RESULTS OBTAINED FROM THE USE OF AN UNPRODUCTIVE SOIL

In this experiment a soil was used which was very unfavorable to plant growth, due to previous annual applications of ammonium sulfate only. Although the pH value of the water extract of this soil was 4.4, the barley seedlings which were planted in it grew fairly well for a time as is indicated by the data of table 7. The soil had about the same water-holding capacity as that

TABLE 6

Effect of a second crop of soybeans upon the oxidation processes, loam soil. Experiment conducted from June 18 to July 19, 1920

	INTERVALS BETWEEN TITRATIONS	CHECK			SOYBEANS		
		Jar 1	Jar 2	Jar 3	Jar 4	Jar 5	Jar 6
	days	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
CO_2 removed from system	0–2	257.8	93.2	176.4	244.4	144.5	116.0
	3–4	296.0	378.9	326.2	365.7	203.0	378.8
	5–6	208.2	208.2	146.6	138.8	159.5	224.5
	7–9	483.0	208.2	465.0	228.7	94.1	109.9
	10–11	341.2	200.9	207.8	256.9	134.3	138.9
	12–14	208.2	179.4	199.0	138.9	131.7	120.2
	15–18	184.7	150.6	165.8	143.2	75.2	143.6
	19–21	208.0	224.9	192.6	217.8	99.0	135.5
	22–24	89.6	91.5	79.2	150.4	93.3	144.6
	25–27	194.3	113.6	139.5	125.2	99.8	144.6
	28–31	185.6	123.4	129.6	162.4	133.5	90.9
Total		2059.1	1972.8	2223.7	2172.4	1367.9	1747.6
Organic matter in crop				59.9	2268.3	1400.0	2066.2
Organic matter in seedlings					155.5	155.5	155.5
Organic matter fixed during experiment				59.9	2112.8	1244.5	1910.7
CO_2 fixed by plants during experiment				106.4	3648.8	2149.3	3299.8
Total CO_2 obtained from soil		2659.1	1972.8	2330.1	5921.2	3517.2	5047.4
Average CO_2 obtained from soil		2320.7 mgm.			4795.2 mgm.		
Average increase over check					106.6%		

used in the preceding experiments. Since it was deficient in organic matter 20 gm. of the prepared humus previously described was added to each 2000 gm. of the soil. The data of table 7 show that the growth of barley even in this soil produced considerable increase in the amount of carbon dioxide recovered as compared with the unplanted checks, although this excess of 29 per cent is not nearly so marked as it is in some of the other experiments. It should be stated here that considerable weed growth appeared in the unplanted cultures and this, in a measure, served to offset the difference in the amounts of carbon dioxide recovered from the planted and unplanted soils.

DISCUSSION

Quantitative measurements of the total carbon dioxide liberated by the oxidation processes taking place in the soil during the period of plant growth have shown that much more rapid oxidation takes place in soil in which plants are growing than in an uncropped soil kept uuder the same conditions of moisture, aeration, temperature, etc. This indicates that the growing roots

TABLE 7

The effect of barley on the oxidation processes in an unproductive soil. Experiment conducted from June 18 to July 19

	INTERVALS BETWEEN TITRATIONS	BARLEY			CHECK (NO PLANTS)		
		Jar 7	Jar 8	Jar 9	Jar 10	Jar 11	Jar 12
	days	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
CO_2 removed from system	0–2	112.6	103.9	88.6	242.1	222.0	193.1
	3–4	83.2	270.6	65.2	240.0	330.0	367.2
	5–7	99.6	134.3	105.3	182.0	290.2	161.0
	8–10	138.9	132.9	101.0	474.0	187.2	361.9
	11–12	112.2	219.9	126.6	249.2	208.2	268.7
	13–15	128.0	121.2	91.9	143.7	168.2	150.7
	16–19	166.0	127.7	92.4	159.3	162.1	206.9
	20–22	108.8	143.5	92.4	158.4	86.2	202.0
	23–25	111.4	194.0	48.2	108.0	136.9	107.1
	26–28	134.9	46.5	129.1	96.7	104.5	117.9
	29–32	128.3	70.0	124.0	98.8	115.6	114.8
Total		1323.9	1563.9	1064.7	2152.2	2011.1	2251.3
Organic matter in crop		1255.5	1358.0	1374.5	*313.8	210.7	147.4
Organic matter in seedlings		217.2	217.2	217.2			
Organic matter produced during experiment		1038.3	1140.8	1157.3	*313.8	210.7	147.4
CO_2 fixed by plants during experiment		1827.4	2007.8	2036.9	552.3	370.3	259.4
Total CO_2 obtained from soil		3151.3	3571.7	3101.6	2704.5	2381.9	2510.7
Average CO_2 obtained from soil		274.9 mgm.			2532.4 mgm.		
Average increase over checks		29.0%					

* Organic matter of weed growth in the unplanted jars.

exert a direct influence upon the oxidation processes taking place in the medium in which they are growing.

It is known, of course, that soil oxidation processes are largely due to biological activity. It is also recognized that such activity results in the production of quantities of available plant nutrients, both directly through the nitrification of soil organic matter, and indirectly through the increased dissolving power of a more highly carbonated soil solution. Thus any stimulus given to the oxidizing processes in the soil would naturally tend to increase the available plant nutrients and this in turn should stimulate plant growth.

That such a stimulus is given to the oxidizing processes in the soil is clearly brought out by the quantitative experimental data above presented. It is therefore reasonable to suppose that a symbiotic relationship may exist between the growing plants and the oxidizing organisms in the soil.

Russell (50) has suggested that growing plants may exert a direct influence upon biological activity taking place in a soil. The present experiments support this suggestion in so far as biological oxidation is concerned. However, the direct cause of the stimulating influence of the growing plants upon the oxidizing processes in the soil is not at all clearly understood and any attempted explanation can only be suggestive. It is well known, of course, that the root hairs which develop on growing roots function only a few days and then die while new ones are continually being formed. Large numbers of other cells and cell aggregates are also continually being sloughed off from the roots growing in the soil.

All of this cast-off material undoubtedly oxidizes very rapidly, although Knudson (26), who studied the viability of root cells detached during plant growth in solution cultures, has shown that such detached cells may remain alive from 40 to 50 days. In the soil, however, the conditions for rapid oxidation are much more favorable and there is good reason to believe that this cast-off organic material decomposes rapidly. All such cast-off organic material which is continually being supplied by the plants during the active growing period apparently furnishes readily available energy to the soil microörganisms involved in the oxidation processes. It is entirely possible that this readily available energy together with excretions from the growing roots may stimulate the oxidizing organisms in such a way as to bring about a more rapid and a more complete decomposition of the previously deposited organic matter. All the experimental evidence here brought forth points to such a conclusion.

SUMMARY

Experiments were conducted to determine the influence of growing plants upon the oxidation activities taking place in soil and specially prepared sand cultures. Quantitative measurements of the total carbon dioxide liberated from the soil or sand cultures were here taken as the index of the oxidation activities. Special apparatus was devised by means of which the total carbon dioxide liberated by the oxidation processes taking place in the substrata employed during the periods of plant growth could be measured quantitatively. This involved the complete exclusion of atmospheric carbon dioxide. That portion of the total carbon dioxide (evolved from the soil) which was fixed by the plants in the photosynthetic processes was determined by analyses of the plants for total carbon. The remainder of the total carbon dioxide was retained in absorption towers containing barium hydroxide and was quantitatively determined.

1. The oxidation processes in sand cultures to which organic matter was added were accelerated by growing green plants, the average total carbon dioxide recovery from the planted cultures being 12.1 per cent higher than that from the unplanted check cultures.

2. Growing plants very greatly increased the oxidation processes in a soil of average productiveness as indicated by the total carbon dioxide produced. The average total amounts of carbon dioxide recovered from cultures planted with buckwheat, field peas, and soybeans were 116.5 per cent, 70.8 per cent, and 60.0 per cent greater, respectively, than were the corresponding amounts recovered from the unplanted check cultures.

3. During the growth of a second crop of soybeans on the same soil, the increase in the oxidation activities over those exhibited in the unplanted soil was more pronounced than it was during the growth of the first crop.

4. All the experimental evidence obtained indicates that growing plants of buckwheat, barley, soybeans, and field peas have a beneficial influence upon oxidation activities in the substrata in which the plants were grown and suggests a symbiotic relationship between the soil-oxidizing organisms and the growing green plants.

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REFERENCES

(1) Association of Official Agricultural Chemists 1916 Method for the determination of organic carbon. *In* Jour. Assoc. Off. Agr. Chem., v. 1, p. 18.

(2) Bedford, Duke of, and Pickering S. W. 1914 The effect of one crop upon another. *In* Jour. Agri. Sci., v. 6, p. 136–151.

(3) Berthelot, M. 1887 Sur la fixation direct de l'azote gazeux de l'atmosphere par les terres végétalés avec le concours de la végétation. *In* Compt. Rend. Acad. Sci. (Paris), v. 104, p. 625–630.

(4) Boussingault, J. B. 1854 Mémoirs de chemie Agricole et de Physiologie, p. 441.

(5) Boussingault, J. B., and Lewy, B. 1853 Mémoir sur les composition de l'air confine dans la terre végétale. *In* Compt. Rend. Acad. Sci. (Paris), v. 35, p. 765.

(6) Brown, P. E. 1912 Bacteriological studies of field soils: II, The effects of continuous cropping and various rotations. *In* Iowa Res. Bul. 6, p. 215–246.

(7) Brown, B. E., and MacIntire, W. H. 1910 Seasonal nitrification, soil moisture and lime requirements in four plots receiving sulphate of ammonia. *In* Penn. Agr. Exp. Sta. Rpt. 1909–10, p. 57–63.

(8) Caron, A. 1895 Landwirtschaftlich bakteriologische Probleme. *In* Landw. Vers. Stat., v. 45, p. 401–418.

(9) Clark, W. M., and Lubs, H. A. 1917 The colorimetric determination of the hydrogen-ion concentration and its application in bacteriology. *In* Jour. Bact., v. 2, p. 1–34, 109–136, 191–236.

(10) CUMMINGS, M. B., AND JONES, C. H. 1918 The aerial fertilization of plants with carbon dioxide. Ver. Agr. Exp. Sta. Bul. 211.

(11) DEHERAIN, P. P. 1897 Sur la composition des eaux de drainage. *In* Compt. Rend. Acad. Sci. (Paris), v. 131, p. 131–193.

(12) EBERHART, M. 1900 Action de l'air sec et de l'air humide sur les végétaux. *In* Compt. Rend. Acad. Sci. (Paris), v. 131, p. 131–193.

(13) FRAPS, G. S. 1908 The production of active nitrogen in the soil. Texas Agr. Exp. Sta. Bul. 106.

(14) FRED, E. B., AND HART, C. B. 1915 The comparative effect of phosphates and sulphates on soil bacteria. Wis. Agr. Exp. Sta. Res. Bul. 35.

(15) GAINEY, P. L. 1919 Parallel formation of carbon dioxide, ammonia, and nitrate in soil. *In* Soil Sci., v. 7, p. 293–311.

(16) GILLESPIE, L. J., AND HURST, L. A. 1917 Hydrogen-ion concentration measurements of soils of two types, Caribou loam and Washburn loam. *In* Soil Sci., v. 4, p. 313–319.

(17) GREAVES, J. E., STEWART, R., AND HIRST, C. F. 1917 Influence of crop, season and water on the bacterial activities of the soil. *In* Jour. Agri. Res., v. 9, p. 293–341.

(18) HARRISON, W. H., AND AIYER, S. 1913 Gases of swamp rice soils. *In* Mem. Dept. Agr. India, Chem. Ser., no. 3, p. 65–106; Ser. 4, p. 1–17.

(19) HEINZE, B. 1910 Bodenbakteriologische Untersuchungen. *In* Landw. Jahrb., v. 39, Ergänzungsband 3, p. 314–343.

(20) HILGARD, E. W. 1906 Method for determination of maximum water capacity. *In* Soils, their Formation, Properties and Composition. p. 208–209. MacMillan, New York.

(21) JENSEN, C. A. 1910 Seasonal nitrification as influenced by crops and tillage. U. S. Dept. Agr. Bur. Plant Indus. Bul. 173.

(22) KELLERMAN, K. F., AND ALLEN, E. R. 1911 Bacteriological studies of the soils of the Truckee Carson irrigation project. U. S. Dept. Agr. Bur. Plant Indus. Bul. 211.

(23) KELLERMAN, K. F., AND WRIGHT, R. C. 1914 Mutual influence of certain crops in relation to nitrogen. *In* Jour. Amer. Soc. Agron., v. 6, p. 204–210.

(24) KING, F. H., AND WHITSON, A. R. 1901 Development and distribution of nitrates and other soluble salts in cultivated soils. Wis. Agr. Exp. Sta. Bul. 85.

(25) KING, F. H., AND WHITSON, A. R. 1902 Development and distribution of nitrates in cultivated soils. Wis. Agr. Exp. Sta. Bul. 93.

(26) KNUDSON, L. 1910 Viability of detached root cap cells. *In* Amer. Jour. Bot., v. 6, p. 309–310.

(27) KOENIG, J. 1907 Einige neue Eigenschaften des Ackerbodens. *In* Landw. Vers. Stat., v. 63, p. 478; v. 66, p. 401.

(28) LADD, E. F. 1901 Humus and soil nitrogen. N. Dak. Agr. Exp. Bul. 47, p. 685–721.

(29) LAWES, J. B., GILBERT, J. H., AND WARINGTON, R. 1883 Nitrogen as nitric acid in the soils and sub-soils at Rothamstead. *In* Jour. Royal Agr. Soc., v. 19, p. 331–367.

(30) LEATHER, J. W. 1912 Records of drainage in India. *In* Mem. Dept. Agr. India, Chem. Ser. 2, p. 63–140.

(31) LECLAIR, C. A. 1916 The influence of the growth of cowpeas upon some physical, chemical and biological properties of soil. *In* Jour. Agr. Res., v. 5, p. 439–447.

(32) LEMMERMAN, O., ASO, K., FISCHER, H., AND FRESENIUS, L. 1911 Untersuchungen über der zersetzung der Rohlenstoffverbindungen verschiedener organischer Substanzen im Boden, speziell unter dem Einfluss von Kalk. *In* Landw. Jahrb., v. 41, p. 217–256.

(33) LIPMAN, J. G. 1910 A method for the study of soil fertility problems. *In* Jour. Agr. Sci., v. 3, p. 297–300.

(34) LIPMAN, J. G. 1912 The associative growth of legumes and non-legumes. N. J. Agri. Exp. Sta. Bul. 253.

(35) LIPMAN, J. G., AND BLAIR, A. W. 1914 The formation of carbon dioxide and nitrates in the presence of large amounts of carbohydrates. *In* N. J. Agr. Exp. Sta. 35th Ann. Rpt., p. 220–221.

(36) LYON, F. L., AND BIZZELL, J. A. 1911 A heretofore unnoted benefit from the growth of legumes. N. Y. (Cornell) Agr. Exp. Sta. Bul. 294, p. 365–374.

(37) LYON, F. L., AND BIZZELL, J. A. 1911 The relation of certain non-leguminous plants to the nitrate content of soils. *In* Jour. Franklin Inst., v. 171, p. 1–16.

(38) LYON, F. L., AND BIZZELL, J. A. 1913 Some relations of certain higher plants to the formation of nitrates in soils. *In* N. Y. (Cornell) Agr. Exp. Sta. Mem. 1, p. 9–109.

(39) LYON, F., AND BIZZELL, J. A. 1913 The influence of alfalfa and timothy on the production of nitrates in soils. *In* Centrbl. Bakt. (etc.), Abt. 2, v. 37, p. 161–167.

(40) LYON, F. L., AND BIZZELL, J. A. 1920 The formation of nitrates in a soil following the growth of red clover and of timothy. *In* Soil Sci., v. 9, p. 53–54.

(41) MCBETH, S. G., AND SMITH, N. R. 1914 The influence of irrigation and crop production on soil nitrification. *In* Centrbl. Bakt. (etc.), Abt. 2, v. 40, p. 24–51.

(42) NEWCOMBE, F. C., AND BOVERMAN, E. A. 1918 The behavior of plants in unventilated chambers. *In* Amer. Jour. Bot., v. 5, p. 284–294.

(43) NELLER, J. R. 1917 The influence of the roots of growing plants upon the activity of soil microorganisms as indicated by the production of carbon dioxide from the soil. *In* N. J. Agr. Exp. Sta. 38th Ann. Rpt., p. 414–415.

(44) NELLER, J. R. 1918 Studies on the correlation between the production of carbon dioxide and the accumulation of ammonia by soil organisms. *In* Soil Sci., v. 5, p. 225–241.

(45) NELLER, J. R. 1920 Oxidizing power of soil from limed and unlimed plots and its relation to other factors. *In* Soil Sci., v. 10, p. 29–39.

(46) POTTER, R. S., AND SNYDER, R. S. 1916 Carbon dioxide production in soils and carbon and nitrogen changes in soils variously treated. *In* Iowa Agr. Exp. Sta. Res. Bul. 39, p. 253–309.

(47) POTTER, R. S., AND SNYDER, R. S. 1918 The production of carbon dioxide by moulds inoculated into sterile soil. *In* Soil Sci., v. 5, p. 359.

(48) RUSSELL, E. J. 1905 Oxidation in soils and its connection with fertility. *In* Jour. Agr. Sci., v. 1, p. 261–270.

(49) RUSSELL, E. J. 1914 The nature and amount of fluctuations in nitrate contents of arable soils. *In* Jour. Agri. Sci., v. 6, p. 18–57.

(50) RUSSELL, E. J. 1917 Soil Conditions and Plant Growth. p. 182. Longmans Green & Co.

(51) RUSSELL, E. J., AND APPLEYARD, A. 1915 The atmosphere of the soil; its composition and the causes of variation. *In* Jour. Agr. Sci., v. 7, p. 1–45.

(52) SCHLOESSING, TH., fils. 1889 Sur l'atmosphère confiné dans le sol. *In* Compt. Rend. Acad. Sci. (Paris), v. 109, p. 618–620; 673–676. (Original not seen.)

(53) SCHREINER, O., AND REED, H. S. 1909 The rôle of oxidation in soil fertility. U. S. Dept. Agr. Bur. Soils Bul. 56.

(54) SCHREINER, O., AND SKINNER, J. J. 1910 Some effects of a harmful organic soil constituent. U. S. Dept. Agri. Bur. Soils Bul. 70.

(55) SCHREINER, O., AND SULLIVAN, M. 1910 Studies in soil oxidation. U. S. Dept. Agric. Bur. Soils Bul. 73.

(56) SHIVE, J. W. 1915 A study of physiological balance in nutrient media. *In* Physiol. Res., v. 1, no. 7, p. 327–395.

(57) SHIVE, J. W. 1918 Toxicity of monobasic phosphates towards soybeans grown in solution cultures. *In* Soil Sci., v. 5, p. 87–122.

(58) STEWART, R., AND GREAVES, J. E. 1909 A study of the production and movement of nitric nitrogen in an irrigated soil. Utah Agr. Exp. Sta. Bul. 106, p. 69–96.

(59) STOKALASA, J., AND ERNST, A. 1905 Ueber den Ursprung die Menge und die Bedeutung den Kohlendioxyde in Boden. *In* Centrbl. Bakt. (etc.), Abt. 2, v. 14, p. 723–736.

(60) TRUOG, E. 1915 Methods for the determination of carbon dioxide and a new form of absorption tower adapted to titrimetric methods. *In* Jour. Indus. Engin. Chem., v. 7, p. 1054.

(61) TURPIN, H. W. 1920 The carbon dioxide of the soil air. N. Y. (Cornell) Agr. Exp. Sta. Mem. 32, p. 319–362.

(62) VAN SUCHTELEN, H. 1910 Ueber die Messung der Lebenstatigkeit der aerobiotischen Bakterien im Boden durch die Kohlensäureproduktion. *In* Centrbl. Bakt. (etc.), Abt. 2, v. 14, p. 45–89.

(63) VORHEES, E. B., LIPMAN, J. G., AND BROWN, P. E. 1907 Some chemical and bacteriological effects of liming. N. J. Agr. Exp. Sta. Bul. 210.

(64) WESTGATE, J. M., AND OAKLEY, R. A. 1914 Percentage of protein in non-legumes and legumes when grown alone and in association in field mixtures. *In* Jour. Amer. Soc. Argon., v. 6, p. 210–115.

(65) WOLLNY, E. 1897 Die Zersetzung der organischen Stoffe und die Humusbildungen, Heidelberg.

(66) Woods, A. F. 1902 Observations on the mosaic disease of tobacco. U. S. Dept. Agr. Bur. Plant Ind. Bul. 18.

PLATE I

FIG. 1. A view of the front or outlet side of the apparatus used to determine the influence of plants upon the oxidation activities in the soil. Twelve barium hydroxide towers were used for recovering carbon dioxide from air removed while soda lime in the long horizontal tube, lying on the table, freed the indrawn air from carbon dioxide. The six central jars contain barley and soybean plants.

FIG. 2. A view of the rear of the apparatus showing the twelve distributing bottles containing dilute sulfuric acid solutions. The circular grooves into which the inverted battery jars are sealed may also be noted.

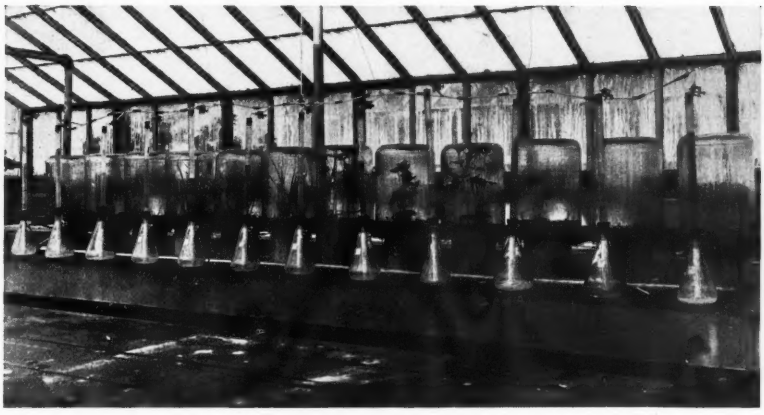

FIG. 1

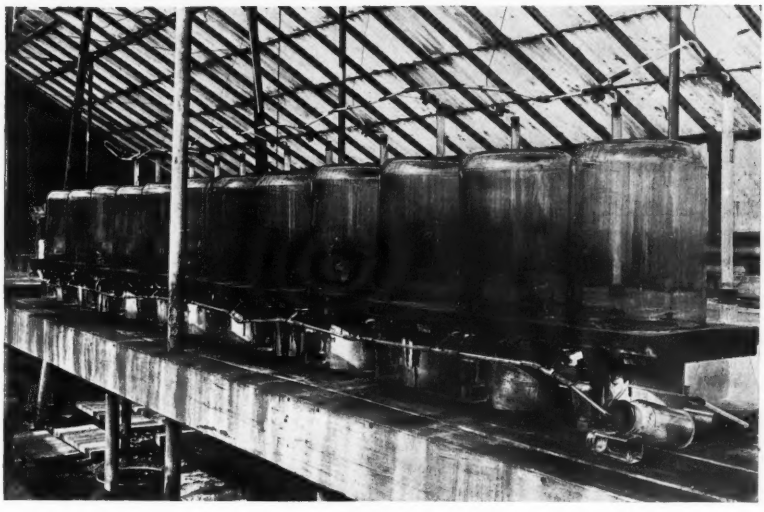

FIG. 2

PRELIMINARY STUDIES ON THE ISOLATION OF SULFUR-OXIDIZING BACTERIA FROM SULFUR-FLOATS-SOIL COMPOSTS[1]

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The most important work on the sulfur cycle through biological processes treats the subject from two aspects. One is the study of different groups and species of organisms which are responsible for the reduction and oxidation processes in the sulfur cycle, and which have been mainly isolated from sulfur springs, canal water, sea slime, swamps, and fresh water. The other one is the study of the oxidation of sulfur in the soil.

Winogradsky (34, 35) in his classical researches described, among others, the important species *Thiothrix* and *Beggiatoa*. According to Winogradsky, the reactions involved in the sulfur transformation by thiobacteria are as follows:

$$2H_2S + O_2 = 2H_2O + S_2 + 122 \text{ calories}$$
$$S_2 + 3O_2 + 2H_2O = 2H_2SO_4 + 282 \text{ calories}$$

The H_2S is oxidized and sulfur is stored by the bacteria in the amorphous form as minute granules. The presence of sulfur in the bacteria may be demonstrated only when an excess of H_2S is present. The sulfur is then oxidized to H_2SO_4 and the process is intracellular. The sulfuric acid is neutralized by carbonates which Winogradsky supplied to the substratum.

$$H_2SO_4 + CaCO_3 = CaSO_4 + CO_2 + H_2O$$

or

$$H_2SO_4 + CaH_2(CO_3)_2 = CaSo_4 + 2\,CO_2 + 2H_2O$$

The *Thiothrix* and *Beggiatoa* were studied later by Keil (17) who confirmed the work of Winogradsky. He described the *Beggiatoa* as forming a white film, the *Thiothrix*, a grayish white film. He pointed out the autotrophic nature of the thiobacteria. Dügelli (8), in his extensive review of the work on the sulfur bacteria places the species *Thiothrix* and *Beggiatoa* as a group of H_2S oxidizing, colorless, threadlike sulfur bacteria. The *Beggiatoa* is described

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as a cylindrical threadlike cell, motile, sometimes attaining a length of 1 cm. The former is also a threadlike form, rod shaped. Both organisms are found in the slime of the sea, and in sulfur springs, but *Thiothrix* is located in the low slime, and *Beggiatoa* in the upper slime. Both of these are accompanied by infusoria, phormidium and oscilatoria. Both consume ammonia salts as a source of nitrogen and carbon dioxide as a source of carbon. Energy is derived from the oxidation of the sulfur.

Winogradsky (34) described a great many species of other sulfur bacteria which belong to the group Rhodobacter. One of the prominent species is *Thiorhodaceen;* these organisms deposit sulfur in their bodies, develop better in the light than in the dark, require less oxygen and are able to stand a higher concentration of H_2S than either the *Thiothrix* or *Beggiatoa*. They were discovered as early as 1826 by Ehrenberg of Jena, who named them *Monas Okenii*. It is reported by Jegunow (13, 14, 15) that the waters of the Black Sea near Odessa are colored red at times, because of the activities of Rhodobacter.

Another group of sulfur organisms very similar to the *Thiothrix* and *Beggiatoa* is mentioned by Düggeli. It is a group of colorless nonthreadlike H_2S oxidizing organisms. Hinze (10) described a member of this group in 1903 and named it *Thiophysa volutans*. Omeliansky (31) also described an organism of this group, *Thiospirillium Winogradskii*. Molisch (29) described *Spirilum granulatum*. The organisms of this group work slower than the *Thiothrix* and the sulfur from their bodies may be obtained as crystals from glycerin solution.

Another very important group of sulfur bacteria is the thiosulfate group. They convert thiosulfates into tetrathionate:

$$3NaS_2O_3 + 50 = 2\,Na_2SO_4 + Na_2S_4O_6$$

They were discovered by Natanssohn (30) and described in 1903. In 1904, Beijerinck (2) confirmed the findings of Natanssohn and names them *Thiobacillus thioparus*. According to the description of Beijerinck, *Thiobacillus thioparus* is a small, thin, short rod, $3 \times 0.5\mu$, non-spore forming and very motile. The organisms are kept with difficulty on solid culture media. The reaction, according to Beijerinck, is:

$$2\,Na_2\,S_2O_3 + O_2 = 2\,Na_2SO_4 + 2S$$

Jacobsen in 1912 (11) worked with these organisms and pointed out that even elementary sulfur is oxidized; however, while he noted the formation of sulfates he also found traces of H_2S. His best cultures oxidized only 12 per cent of the sulfur in 8 weeks. The isolations were made from sweet and sea water. Jacobsen (12) also worked with the sulfur organisms that oxidize H_2S.

Another group of sulfur organisms should be mentioned in this brief review, namely, denitrifying sulfur bacteria. Beijerinck (2) discovered them in

1904 and later in 1912 Lieske (18) studied their physiological activities. They are known as *Thiobacterium denitrificans*. They thrive in the absence of free oxygen and derive it from nitrates, liberating nitrogen. They may use either sulfur or thiosulfates. The reactions involved are as follows:

$$5S + 6KNO_3 + 2CaCO_3 = 3K_2SO_4 + 2CaSO_4 + CO_2 + 2N_2$$

or

$$5Na_2S_2O_3 + 8KNO_3 + 2NaH\,CO_3 = 6Na_2SO_4 + 4K_2SO_4 + 2CO_2 + 4N_2 + H_2O$$

The work of Gicklehorn (9) should be mentioned at this point. He claims to have studied a number of new species of sulfur organisms, some even from garden soil. His work was mostly carried out with impure cultures and only superficial morphological characters are described.

When we turn out attention to the process of sulfur oxidation in the soil, we find that very little has been done from the standpoint of microbiological elements involved. All of the studies, with few exceptions, were on the fertilizing action of sulfur. It must be kept in mind that sulfur oxidation in soils, while chiefly a biological phenomenon, may be, in part, the product of chemical reactions. This has been pointed out by Kappen and Quensell (16). However, the experiments of Boullanger (3) and Demolon (6) and Lipman and McLean (23, 24, 28) emphasized the importance of the biological factor. They proved that in sterilized soils the sulfur applied was not converted into sulfates as efficiently as in non-sterilized soils. Brioux and Guerbet (4) studied the transformation of sulfur in the soil with special reference to the oxidation of sulfur and the effect of carbohydrates, peptones and other nitrogenous materials on the process.

Lint (19, 20, 21) studied the rate of oxidation of sulfur. Shedd (32) made a comparative study of sulfur oxidation in soil and sand. Brown and Kellog (5) endeavored to determine the oxidizing power of different soils. Vermorel and Dantony (33) studied the oxidation of sulfur and iron sulfide in relation to growth of wheat and beans. Ames and Boltz (1) studied the oxidation of sulfur and its effect on availability of phosphates under anaerobic conditions and, of course, found no oxidation. This point was brought out forcibly in the work of Lipman and his co-workers. The importance of their work cannot be overemphasized. It brings the problem of sulfur oxidation to a point where both scientific and practical investigations should be followed. The work will not be reviewed here and those interested may consult the original papers.

The work reported here is an outgrowth of the investigations of Lipman. The purpose of these experiments was to study the process of sulfur oxidation, the reactions involved, the isolation of the organisms responsible for the oxidation and a study of their morphological and physiological characters.

EXPERIMENTAL PROCEDURE

A culture medium was prepared with the following composition:

	gm.
K_2HPO_4	1.0
$MgSO_4$	0.5
KCl	0.5
$FESO_4$	trace
$NaNO_3$	2.0
$CaCO_3$	10.0
Sugar	5.0
Sulfur	10.0
	cc.
Distilled water	1000

This medium was called medium 1. Another one, medium 2, was of the same composition without calcium carbonate. The medium was distributed into 250-cc. Erlenmeyer flasks and sterilized under pressure for twenty minutes. This method of sterilization had to be abandoned since it caused the sulfur to cake. Intermittent sterilization for $\frac{1}{2}$ hour on three consecutive days was finally adopted as the best method. Six flasks of each medium were inoculated with 0.1, 0.2, 0.3, 0.4, 0.5, and 1.0 gm. of a soil-sulfur-floats compost known for its active sulfur-oxidizing power. The cultures were incubated at 25°C. Observations were made daily and after three or five days remarkable activity could be noticed in medium 1. Gas was bubbling through the medium which was ascribed to the action of sulfuric acid on the carbonate. To test the gas evolved, slight suction was applied to several flasks. Before entering the culture flasks the air was freed from CO_2 by means of concentrated potassium hydroxide solution. The CO_2 in the air withdrawn from the flasks was caught in barium hydroxide solution. The formation of barium carbonate proved that CO_2 was evolved. The degree of cloudiness served, in a way, as a measure of the activities of the organisms. The hydrogen ion concentration of both media was determined. In some flasks containing medium 2 the pH value went down to 3.0 after twelve days, while in medium 1 it was 4.8. Upon continuous transfer the activity ceased and no gas was evolved in medium 1, and in medium 2 the pH value would not go below 4.0. The original pH of media 1 and 2 were 8.2 and 6.0, respectively. Thus some oxidation must have taken place, although the lowering of the pH could be a result of the formation of organic acids from the sugar.

Plates from the same media with 1.5 per cent agar were poured and a number of bacteria and fungi were isolated. Microscopic examinations showed the presence of cocci and bacilli. The latter were different sized rods, motile and non-motile, large and very small. The representative types were inoculated on liquid medium 2. In no case did the pH value reach a point lower than 4.4 and, since some flasks with medium 2 inoculated with crude cultures gave a pH as low as 3.0 (no indicators were on hand at that time for the pH below

3.0), it was concluded that the more strongly oxidizing organisms did not appear on the plates.

The failure of the cultures to show activity upon transfer into original media showed that these media, especially medium 1, were not satisfactory. The task at hand, then, was to get a medium which would allow good growth and at the same time give an index of the growth of the organisms.

EXPERIMENT 2

Medium 3 was prepared from the following materials:

$MgCO_3$	0.5
K_2HOP_4	1.0
KCl	0.5
$FeCl_3$	trace
$NaNO_3$	2.0
Sugar (Mercks)	10.0
Sulfur	10.0
	cc.
Distilled water	1000

This was inoculated in the same way as media 1 and 2. Close observations were made on the flasks and the same phenomenon as in the case of medium 1 was noticed. After 15, 25, 40, 55 and 70 and, in some series, 90 days of incubation, quantitative tests were made for the production of sulfates. The results are presented in table 1.

TABLE 1

Formation of SO_4 in cultures with different amounts of inoculum

AGE OF CULTURE	0.1 GM. INOCULUM		0.2 GM. INOCULUM		0.3 GM. INOCULUM		0.4 GM. INOCULUM		0.5 GM. INOCULUM		1.0 GM. INOCULUM	
	Reaction	SO_4 per 100 cc.	Reaction	SO_4 per 100 cc.	Reaction	SO_4 per 100 cc.	Reaction	SO_4 per 100 cc.	Reaction	SO_4 per 100 cc.	Reaction	SO_4 per 100 cc.
Series 1												
days	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*
15	5.8	42.2	6.0	36.3	6.8	4.0	5.8	40.6	5.6	49.2	5.2	64.7
25	5.4	96.4	5.6	79.4	6.6	5.8	5.4	86.7	5.2	89.4	4.8	96.3
40	4.8	124.4	4.8	116.8	6.4	4.6	4.6	134.7	4.8	140.6	4.6	138.4
55	3.8	168.0	3.6	148.4	6.0	8.2	3.4	176.4	3.6	181.2	3.4	192.1
70	3.0	204.2	3.0	194.6	6.2	6.0	3.0	212.3	3.0	214.2	3.0	218.3
Series 2												
10	6.2	4.6	6.6	3.6	6.6	3.6	6.6	3.4	5.8	63.7	5.8	61.6
20	5.6	64.2	6.4	4.0	6.4	4.7	6.6	4.1	5.4	74.9	5.6	76.3
30	5.2	70.2	5.8	6.2	6.0	6.1	6.2	5.0	5.0	82.1	5.2	81.7
50	4.2	139.4	6.0	5.6	5.8	4.8	6.4	4.6	3.8	128.9	4.6	117.4
70	3.4	174.6	5.8	4.8	6.2	5.9	6.2	4.2	3.0	163.9	3.4	152.6
90	3.0	192.6	6.2	3.8	6.0	4.8	6.0	4.2	3.0	191.6	3.0	179.8

It will be noticed that the results are not uniform. In some cases no oxidation could be detected. Even those that showed maximum oxidation did not oxidize more than 8 or 9 per cent of the sulfur. Dextrose was substituted for sucrose, but with no effect. On the contrary a reduction process would go on for a while and then cease. It was ascribed then to the reducing power of the dextrose, but later it was found that even with sucrose the same thing occurred, only to a smaller degree.

The results reported in table 1 are typical of many series. In some cases even 0.5 gm. of inoculum did not give any sulfates. In the course of these two experiments numerous fungi, especially a mucor, and a fusarium accompanied the cultures. It was thought then, that the fungi participated in the early stages of the sulfur oxidation, which the bacteria continued.

Since after the second or third transfer the cultures generally deteriorated in the same way as with media 1 and 2, another set of experiments was started.

EXPERIMENT 3

It was pointed out in experiment 2, that a reducing reaction was observed when 1 per cent of dextrose or even sucrose was used in the medium. It seemed logical to conclude that in the steps involved in the production of sulfates, the reduction process is an undesirable side reaction, since in the end the H_2S formed is oxidized. Medium 3 had no sulfates to start with and, for the production of H_2S, some of the sulfur has to be oxidized first and then reduced only to be oxidized again. Therefore, the elimination of the reduction process was desirable. Since no H_2S formation was observed in experiment 1 and since the primary difference between media 1 and 3 was the carbohydrate content, a medium was prepared with 0.1 per cent dextrose and another without dextrose. It was recognized that the sulfur-oxidizing organisms in the soil may also be of autotrophic nature. In most experiments there was increased oxidation of sulfur, in medium 3 with the low dextrose content. The autotrophic nature of the sulfur oxidizing organisms in the soil was therefore tested. Cultures with medium 3 were prepared without dextrose, inoculated and incubated. The results in table 2 show that the sulfur-oxidizing organisms in the soil do not use any carbohydrate for energy and body building, but may derive their carbon needs from carbonates or CO_2 in the air, and their energy from the oxidation of the sulfur. The dextrose flasks, however, showed more oxidation than the non-dextrose. It was then ascribed fully to the stimulation of the dextrose. While this may be true it is now known from the work of Lipman, Waksman and Joffe (27), that the reaction plays an important rôle. It is therefore possible that in the dextrose flasks where fungi readily developed, the reaction favored the real sulfur oxidizers. Upon continuous transfer in the dextrose-free media activity ceased, the reaction would not go down and no oxidation would take place. On the other hand, the dextrose medium gave better results and the cultures were transferred continuously from 25-, 50-, and 90-day cultures with satisfactory results.

In many instances the cultures deteriorated but in two cultures which were freed from all the fungi except the fusarium, the oxidation of sulfur reached the 18 per cent mark. The pH was below 2.0. Attempts to continue the work with that particular culture on the same medium failed. Further modifications were necessary. The work of Lipman and McLean (23, 28)

TABLE 2

*Sulfates produced in the presence or absence of dextrose**

AGE OF CULTURE	WITH DEXTROSE				WITHOUT DEXTROSE			
	First transfer		Second transfer		First transfer		Second transfer	
	Reaction	SO_4 per 100 cc.	Reaction	SO_4 per 100 cc.	Reaction	SO_4 per 100 cc.	Reaction	SO_4 per 100 cc.
Series 1								
days	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*
10	6.8	6.4	6.2	8.6	6.2	8.6	6.8	4.6
20	6.6	4.6	5.4	38.4	6.0	10.3	6.4	8.4
30	6.0	8.4	5.0	42.3	5.4	22.7	6.4	6.8
40	5.4	16.4	4.6	58.6	5.2	40.6	6.2	6.2
50	5.0	28.8	4.0	86.4	4.6	73.8	6.0	10.2
60	4.6	42.8	3.2	98.4	4.0	94.7	5.8	21.6
70	3.2	80.1	3.0	146.4	3.6	128.7	5.0	38.9
80	3.2	96.6	3.0	184.6	3.0	198.4	4.8	42.7
90	3.2	101.2	2.6	288.6	3.0	238.6	4.4	59.6
Series 2								
	WITH DEXTROSE				WITHOUT DEXTROSE			
	Second transfer		Third transfer		Original inoculation		First transfer	
10	6.8	5.8	6.4	7.9	6.0	12.4	6.0	16.4
20	6.4	7.6	5.2	40.3	5.2	43.7	5.8	18.4
30	5.8	14.9	4.8	59.6	4.8	76.4	5.0	46.9
40	4.6	60.4	4.0	94.6	4.0	101.3	4.6	70.3
50	4.2	92.4	3.0	186.1	3.4	176.4	4.4	84.6
60	4.0	116.7	2.6	270.0	3.0	242.6	4.0	112.8
85	3.6	164.8	2.2	328.4	2.6	306.9	3.6	151.7

* 0.2 gm. inoculum was used.

with sulfur-floats-soil composts showed an inhibitory effect of sodium nitrate on the oxidation of sulfur. This suggested the elimination of sodium nitrate as a source of nitrogen and ammonium chloride was substituted.

EXPERIMENT 4

In the course of the work, repeated attempts to plate out the cultures on the respective media with agar, in the early stages of incubation, gave mostly fungi with the mucor and fusarium mentioned and also some bacterial colonies.

Transfers of the bacteria from the plates into the liquid media either gave no oxidation, or in two cases only slight oxidation. In no case did the pH go down lower than 4.4.

Platings in the advanced stages gave no bacterial colonies and only the fusarium. Microscopic examinations of the old cultures showed the presence of very minute bacteria, rod-shaped with rounded ends. The organisms would not grow on solid media. Gypsum blocks, silica jelly, washed agar gave no results. At this point media 15 and 16 with ammonium chloride as a source of nitrogen instead of sodium nitrate, were prepared.

MEDIUM 15		MEDIUM 16	
	gm.		*gm.*
K_2HPO_4	0.5	K_2HPO_4	0.5
NH_4Cl	2.0	NH_4Cl	2.0
Dextrose	0.1	Dextrose	0.1
$MgCO_3$	0.25	$MgSO_4$	0.5
Sulfur	10.0	Sulfur	10.0
	drops		*drops*
$FeCl_3$, 10 per cent solution	2–3	$FeCl_3$, 10 per cent solution	2–3
	cc.		*cc.*
Distilled water	1000	Distilled water	1000

As in the early part of the work, the medium was distributed in 250-cc. Erlenmeyer flasks. Inoculations were made from the original sulfur-floats-soil composts and also from the best culture from experiment 3, where the pH was below 2.0. After several preliminary experiments medium 16 was adopted as the best. In the early stages of the cultures, the fungi would appear on plating. As in the earlier work, the mucor and fusarium predominated. Aspergilli and penicillia were also present, but on long incubation and transfer they disappeared, leaving only the minute bacteria and in some cases the fusarium. Table 3 gives the results. It will be noticed that in the third transfer, the oxidation of sulfur was very rapid after the reaction reached pH of 2.0. Attempts were made to adjust the reaction with sulfuric acid, but the work in this direction was of a preliminary nature and failed. However, later the importance of the reaction of the medium was demonstrated. An analysis of the sulfur left showed that 48 per cent had been oxidized. One cc. of the liquid required 8.3 cc. of 0.1N NaOH for neutralization. Further transfers from the 150-day culture upon other media as reported in the work of Lipman, Waksman and Joffe (27) proved the purity of the culture.

Mention was made in experiment 1 about the isolation of bacteria which brought down the reaction of the media to a pH 4.4. These organisms readily grow on the solid Czapeck's synthetic medium. They are small rods, non-motile, and showed only the Brownian movement. On Czapeck's slants they make a fairly good growth which is spreading, somewhat raised, odorless and of a slimey consistency. Two organisms were selected which did not differ

TABLE 3

Sulfates produced with NH_4Cl as a source of nitrogen

AGE OF CULTURE	ORIGINAL INOCULATION 0.2 GM. INOCULUM		FIRST TRANSFER FROM 30-DAYS-OLD CULTURE		SECOND TRANSFER FROM 25-DAYS-OLD CULTURE		THIRD TRANSFER FROM 25-DAYS-OLD CULTURE		
	Reaction	SO_4 per 100 cc.	Reaction	SO_4 per 100 cc.	Reaction	SO_4 per 100 cc.	Reaction	SO_4 per 100 cc.	Titratable acidity, $\frac{N}{10}$ NaOH required to neutralize 1 cc. culture
days	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*cc.*
10	4.4	63.6	4.2	84.2	4.0	92.4	4.2	102.6	
15	4.2	78.9	3.8	140.6	3.8	138.6	3.6	146.7	
20	4.0	136.4	3.6	192.4	3.4	198.4	3.2	216.8	
25	3.8	151.2	3.0	246.8	3.0	238.7	3.0	242.6	
30	3.6	174.7	2.8	309.6	2.6	316.4	2.8	308.6	
40	3.0	248.6	2.6	336.4	2.4	354.6	2.4	364.5	
50	2.8	300.2	2.2	400.6	2.0	489.9	2.0	469.7	
60			2.0	584.6			1.8	689.7	
70			1.8	628.8			1.6	792.6	
80							1.4	927.3	
90							1.4	984.6	
100							1.2—*	1016.8	2.75
120							1.2—	1203.6	4.80
150							1.2—	1381.4	8.30

* No indicators were available to measure the pH below 1.2.

morphologically but did differ quite distinctly physiologically. Their laboratory numbers were cultures 5 and Z. Culture 5 had the property of liquefying gelatin. Besides these bacteria, the mucor and fusarium mentioned were also selected to try out their oxidizing power. The amount of phosphate made soluble from floats in liquid culture was taken as a measure of the oxidizing power. The medium used had the following constituents:

	gm.
K_2HPO_4	1.0
$MgSO_4$	0.5
KCl	0.5
$FeSO_4$	0.01
$(NH_4)_2SO_4$	2.0
Floats	10.0
Dextrose	1.0
Sulfur	10.0
	cc.
Distilled water	1000

The medium was placed in flasks and sterilized in the same manner as those used in the early part of the work. Inoculation was made from slants and incubated at 27°C. Table 4 indicates the results.

The lowering of the reaction to pH 2.6 by culture 5 looked suspicious and, although no contamination could be observed microscopically, and the bacterium was again obtained on plates with ordinary agar, it is likely that some other culture accompanied it. The results with the fungi are remarkable. The cultures were kept for over six months and the reaction did not go down any lower than pH 2.4. That they are able to oxidize sulfur could be seen from the fact that 40 per cent of the insoluble phosphates went into solution. It is possible that the process of sulfur oxidation in the soil is a result of associated activities of different forms of microörganisms. This opinion has been repeatedly expressed by Dr. Lipman, and it seems to hold true.

TABLE 4

Phosphorus made available by different microorganisms

AGE OF CULTURE	REACTION				
	Mucor	Fusarium	Culture 5	Culture Z_n	Combination of all four cultures
days	*pH*	*pH*	*pH*	*pH*	*pH*
8	6.0	6.0	6.5	6.4	4.4
18	3.2	4.4	6.2	5.8	4.4
25	3.0	3.0	3.8	6.0	4.2
50	2.6	2.6	2.6	4.2	3.0
*Phosphorus in 100 cc. of medium at the end of incubation.**					
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
	86.1	89.4	58.29	25.2	97.4

* The total amount of P in medium was 153 mgm., of which 26.4 mgm. were in solution to start with.

SUMMARY

1. The sulfur cycle from a biological standpoint has been studied from two aspects: (*a*) oxidation and reduction by sulfur bacteria from canal water, sea slime, sulfur springs, etc., and (*b*) oxidation of sulfur in soils.

2. The more important of these studies are briefly reviewed.

3. The work on the isolation and studies of the sulfur-oxidizing organisms in soils is reported.

4. The presence of a specific organism which in one hundred and fifty days oxidized close to 50 per cent of the sulfur is demonstrated and its purity apparently proven.

5. Associative action of fungi and bacteria in the oxidation of sulfur in soils is discussed.

REFERENCES

(1) AMES, J. W., AND BOLTZ, G. E. 1916 Sulfur in relation to soils and crops. Ohio Agr. Exp. Sta. Bul. 292.

(2) BEIJERINCK, M. W. 1904 Über die Bakterien, welche sich in Dunkeln mit Kohlensauren als Kohlenstoffquelle ernähren Können. *In* Centbl. Bakt. (etc.), Abt. 2, v. 11, p. 593–599.

(3) BOULLANGER, E. 1912 L'action du soufre en fleur sur la vegetation. *In* Compt. Rend. Acad. Sci. (Paris), v. 154, no. 6, 369–370.

(4) BRIOUX, CH., AND GUERBET, M. 1913 Évolution du soufre dans le sol, étude sur son oxydation. *In* Compt. Rend. Acad. Sci. (Paris), v. 156, no. 19, p. 1476–1479. Ann. Sci. Agron., v. 4, s. 2, p. 385–396.

(5) BROWN, P. E., AND KELLOGG, E. H. 1914 Sulfofication in soils. Iowa Agr. Exp. Sta. Res. Bul. 18.

(6) DEMOLON, H. 1913 Recherches sur l'action fertilisante du soufre. *In* Compt. Rend. Acad. Sci. (Paris), v. 156, no. 9, 725–728.

(7) DEMOLON, H. 1912 Sur l'action fertilisante du soufre. *In* Compt. Rend. Acad. Sci. (Paris), v. 154, no. 8, p. 524–526.

(8) DÜGGELI, M. 1919 Die Schwefelbakterien. Neujahrblatt der Naturforschenden Gesellschaft in Zürich.

(9) GICKLEHORN, J. 1920 Über neue farblose Schwefelbakterien. *In* Centbl. Bakt. (etc.), Abt. 2, v. 50, p. 415–427.

(10) HINZE, G. 1903, 1913 Thiophysa volutans, ein neues Schwefelbakterien. *In* Ber. Deut. Bot. Ges., v. 21, p. 309–316; v. 31, p. 189–202.

(11) JACOBSEN, H. C. 1912 Die Oxidation von element. Schwefel durch Bakterien. *In* Folia Microbiol., no. 4, p. 487–496.

(12) JACOBSEN, H. C. 1914 Die Oxidation von Schwefel-Wasserstoff durch Bakterien. *In* Folia Microbiol., no. 3.

(13) JEGUNOW, M. 1896 Bakterien-Geselschaften. Centbl. Bakt. (etc.), Abt. 2, v. 2, p. 11–21, 441–449, 478–482, 739–752.

(14) JEGUNOW, M. 1897 Zur mechanischen Analyse der Bakterienplatten. *In* Centrbl. Bakt. (etc.), Abt. 2, v. 3, p. 467–477.

(15) JEGUNOW, M. 1898 Platten der roten und der δ-Schwefelbakterien. *In* Centrbl. Bakt. (etc.), Abt. 2, v. 4, p. 257–265.

(16) KAPPEN, H., AND QUENSELL, E. 1915 Über die Umwandlungen von Schwefel und Schwefelverbindungen im Ackerboden. *In* Landw. Vers. Stat., v. 86, p. 1–34.

(17) KEIL, F. 1912 Beiträge zur Physiologie der farblosen Schwefelbakterien. *In* Beitr. Biol. Pflanz., v. 11, p. 335–372.

(18) LIESKE, R. 1912 Untersuchungen über die Physiologie denitrifizierender Schwefelbakterien. *In* Ber. Deut. Bot. Gesell., v. 30, p. 12–22.

(19) LINT, H. C. 1914 Report of potato scab experiments. *In* N. J. Agr. Exp. Sta. 36th Ann. Rpt., p. 477–478.

(20) LINT, H. C. 1914 The influence of sulfur on soil acidity. *In* Jour. Indus. Engin. Chem., v. 6, p. 747.

(21) LINT, H. C. 1916 Report on potato scab experiments. *In* N. J. Exp. Sta. 36th Ann. Rpt., p. 375–381.

(22) LIPMAN, J. G. 1916 Sulfur on alkali soils. *In* Soil Sci., v. 2, no. 3, p. 205.

(23) LIPMAN, J. G., AND MCLEAN, H. C. 1918 Experiments with sulfur phosphate composts conducted under field conditions.

(24) LIPMAN, J. G., AND MCLEAN, H. C. 1917 Vegetation experiments on the availability of treated phosphates. *In* Soil Sci., v. 4, no. 4, p. 337–342.

(25) LIPMAN, J. G., MCLEAN, H. C., AND LINT, H. C. 1916 Sulfur oxidation in soils and its effect on the availability of mineral phosphates. *In* Soil Sci., v. 2, no. 6, p. 499–538.

(26) LIPMAN, J. G., MCLEAN, H. C., AND LINT, H. C. 1916. The oxidation of sulfur in soils as a means of increasing the availability of mineral phosphates. *In* Soil Sci., v. 1, no. 6, p. 533–539.

(27) LIPMAN, J. G., WAKSMAN, S. A., AND JOFFE, J. S. 1921 The oxidation of sulfur by microörganisms. *In* Soil Sci. (soon to appear).

(28) MCLEAN, H. C. 1918 The oxidation of sulfur by microörganisms in its relation to the availability of phosphates. *In* Soil Sci., v. 5, p. 251–290.

(29) MOLISCH, H. 1912 Neue farblose Schwefelbakterien. *In* Cent. Bakt. (etc.), Abt. 2, v. 33, p. 55–62.

(30) NATHANSOHN, 1902 Über eine neue Gruppe von Schwefelbakterien und ihren Stoffwechsel. *In* Mittlil. a. d. Zoolog. Stat. zu Neapel, v. 15, no. 4, p. 655.

(31) OMELIANSKI, W. 1905 Über eine neue Art farbloser Thiospirillen. *In* Cent. Bakt. (etc.), Abt. 2, v. 14, p. 769–772.

(32) SHEDD, O. M. 1914 The relation of sulfur to soil fertility. Ky. Agr. Exp. Sta. Bul., 188, p. 595–630.

(33) VERMOREL, V., AND DANTONY, E. 1913 Contribution a l'étude du soufre et des pyrites de fer employes comme engrais. *In* Engrais, v. 28, p. 1304–1306.

(34) WINOGRADSKY, S. 1888 Beiträge zur Morphologie und Physiologie der Bakterien I, Schwefelbakterien, Leipzig.

(35) WINOGRADSKY, S. 1883 Recherches sur les sulfubacteries. *In* Ann. Inst. Pasteur, 3d year, no. 2.

THE EFFECT OF DRYING SOILS ON THE WATER-SOLUBLE CONSTITUENTS[1]

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INTRODUCTION

The effect of drying and of burning soil on the growth of crops was noted in the field by the Roman farmer many years before the beginning of the science of soils. At first, improvement in the crop was attributed to the well known effect of drying on the physical condition of medium and fine-grained soils; later, as chemistry developed, the increased growth was considered to be due to some chemical change which occurred in the soil during the process of drying; while a more recent idea is that the improvement is brought about by readjustment in the microscopic life of the soil.

For several decades greenhouse growers have practiced heating the soil to kill various plant and animal enemies of the crop. In many instances the improvement in growth was greater than could have been affected by sterilization alone, the rank growth of stem and dark green color of leaf being particularly noticeable. Some growers have found it necessary to withhold a portion of the nitrogen usually supplied, to avoid excessive growth of the vegetative portion of the plant and thus permit proper fruiting.

It is the purpose of this paper to review the observed effects of drying and heating soils, and to present some experimental data indicating the effect of drying, and of heating at 105°C., on the amount of total water-soluble solids recovered by extraction with distilled water.

REVIEW OF LITERATURE

Davy (17) says:

"The improvement of lands by burning was known to the Romans. It is mentioned by Virgil in the first book of the Georgics. It is a practice still much in use in many parts of these islands; the theory of its operation has occasioned much discussion, both amongst scientific men and farmers. It rests entirely upon chemical doctrines; and I trust I shall be able to offer you satisfactory elucidations on the subject."

Also:

"When clay or tenacious soils are burnt, ... they are brought nearer to a state analogous to that of sands. . . . . All poor siliceous sands must be injured by it (burning), and here practice is found to accord with the theory."

[1] A thesis submitted to the Faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, September, 1920.

Warington (102) working with soils that had been dried at 55°C. for 8 hours found that the first 150 cc. of extract from 7 pounds of dry powdered soil, contained all the chlorides and 98.8 per cent of the nitrates. He states that, if the soil were wet, a much greater volume of water would be required to leach out all the chlorides and nitrates as it would be necessary to displace all the water present in the soil. He noted that oven-drying caused a reduction in quantity of nitrates, that the decrease was not so great when the soil was dried slowly and that, when air-dried at 10°C., there was an increase in nitrates.

Frank (24) extracted 30 gm. of soil with 2 liters of distilled water, comparing unheated, air-dry soil with the same heated in an autoclave at 100°C. Heating increased the total soluble matter in sand 50 per cent and in the swamp soil over 150 per cent. The soluble organic matter was almost trebled in each case. Larger crops of oats and yellow lupines were produced on the heated soils.

Liebscher (60) found that heating soil with steam increased the solubility of phosphorus and nitrogen compounds.

Schmoeger (88) heated moor soil at 150 to 160°C. for 10 hours, with the result that the phosphorus soluble in hydrochloric acid was doubled.

Deherain and Demoussy (18) heated two soils in an autoclave at 120°C. for 1 hour. When these heated soils were inoculated with fresh soil, they produced more nitrate nitrogen and ammonia than did the original soils.

Pfeiffer and Franke (73) heated soil under a pressure of 1 atmosphere for 3 hours. The soil so treated produced a larger crop of mustard than the unheated soil and it contained a higher percentage of nitrogen.

On heating a garden soil in an oven at 100°C., on 3 successive days, Richter (82) found that the amount of water-soluble organic matter trebled, and the total soluble matter almost doubled.

Tacke (100) showed that a fresh swamp soil contained very little water-soluble phosphorus, and drying at 70 to 80°C. rendered more than half of the total phosphorus soluble in water.

Tacke and Immendorf (101) found the solubility of phosphorus and potassium in some swamp soils was increased by drying at 80°C. In another experiment they found heating at 100 and 180°C. doubled and trebled, respectively, the amount of water-soluble phosphorus.

Stone and Smith (99) report that heating soil improves the color and causes an acceleration of growth of lettuce, cucumbers and tomatoes and that saprophitic fungi not found in unheated soil grew profusely on heated soil, indicating a change in the organic matter.

Krüger and Schneidewind (58) showed definitely that soluble nitrogen and phosphorus was greatly increased by heating. On both unmanured soil and that supplied with sodium nitrate, the growth of mustard was nearly doubled by heating the soil before planting.

Deitrich(19) heated garden soil and secured increased crops; but, curiously enough, pasture soils did not respond in the same way.

Whitney and Cameron (103) found that air-dried soils, in general, had more soluble phosphoric acid, nitric acid, calcium and potassium than fresh soils, and that with few exceptions oven-dried soils had still greater quantities of these materials in soluble form. Nitric acid was most variable.

Card and Blake (9) report in each of two trials, a decrease in yield of lettuce due to soil sterilization, while in one trial radishes gave an increase where nitrate of soda was added to sterilized soil.

Hassenbaümer, Coppenrath, and König (29) report that the solubility of inorganic constituents was increased when 250 gm. of soil and 3 liters of water were heated together under a pressure of 3 atmospheres for 3 hours.

King (45) reviewed fully the recorded experimental work on water extracts of soils and extraction with dilute acids. These reports date back to the work of Grouven in 1858 and include that of Wunder and Eichhorn, 1860, Peters, 1860, Jarriges, 1861–1862, Hoffman, 1863, Schulze, 1864, and Hayden, 1865. Nearly all of these investigators report potash,

lime, magnesia, soda, oxide of iron and aluminum, phosphoric, sulfuric and carbonic acids, chlorine, silica and organic matter separately. Their results vary greatly for any one constituent because of the wide range of soils, temperatures and moisture contents used.

These results are especially interesting since several of them were obtained by methods essentially similar to those of King, who used 100 gm. of soil and 500 cc. of distilled water. The soil was stirred in a mortar with enough water to make a thick paste in order to break down all granules, after which the remainder of the 500 cc. of water was added. Then the supernatant, turbid liquid was transferred to a pint Mason jar and, usually within 15 minutes, to the Pasteur-Chamberland filter chamber.

Filtration was accomplished by a pressure of 30 to 40 pounds. Clear extracts were obtained in 5 to 20 minutes, depending on the type of soil, and the amount of clay and fine silt remaining in suspension to coat the walls of the filters. It was during the 3 minutes of active agitation that the main part of actual solution occurred. It was found that longer washing did not materially increase the amount of salts going into solution. At first the electrical-resistance method was employed for determining concentration but it was found more accurate to evaporate definite quantities of the extract, dry in an oven and weigh. NO_3, HPO_4, Cl and SiO_3 were determined by methods described by Whitney and Cameron (103). Comparisons were made of the salts that could be recovered from fresh soil, soil quickly sun-dried and from that oven-dried at 110°C. Eight soils in four 1-foot sections were used. In the surface foot, of four soils the oven-dry soil had more nitrates, while in the other four, the fresh, moist soil had more; but in the second, third, and fourth sections the nitrates were increased 108, 134 and 61 per cent, respectively. In two of the eight soils the fresh sample showed considerably more HPO_4 in the first section than the dry and in eight instances in the other 3 feet out of the 24 possible cases the fresh soil was slightly higher. In every case (except one in fourth foot), the dry soil gave up much more SO_4; for the eight soils the average increase for the four sections was 265, 310, 281 and 79 per cent, respectively. In one instance only was the fresh soil significantly higher in HCO_3 while in the others the dry was from 48 to 73 per cent higher. Silica was 588, 322, 237 and 236 per cent higher in the oven-dried soil in the four sections. Chlorine was the only element that, on the average, was recovered in smaller quantity from the dry soil. The other acid radicals ran from 1.26 times as much nitrates up to 6.58 times as much of silica in the dry as in the fresh soil.

Later, determinations were made of potash, lime and magnesia in the extract of fresh and oven-dried soils. In part II, King reports good correlation between quantity of soluble salts found, especially HPO_4, and crop yields for the different soil types under investigation.

King concluded that in oven-drying the last of the moisture, for a time at a temperature near the boiling point, increases the solubility of salts and might be expected also to render the organic matter more soluble. He also concluded that when a soil dries its salts are deposited as crystals on the soil particles and salts within the granules are left on the exterior. As the soil is stirred in water, these salts go into solution readily. On the other hand, in a moist soil the solution is simply diluted by adding water and the dissolved salts are disseminated through it in part by diffusion, a slow process. The solution from the dry soil is removed from it before readsorption occurs. Thus, he explains the recovery of more soluble material from the dry than the moist soil.

Hilgard (30) considered the unusual productiveness of desert soils when properly watered, due to an abundant supply of plant nutrients rendered available by the intense heating to which these soils are subjected during the warm season. With King he believed that the soluble salts, on drying, are deposited on the surface of the particles whence they may be "readily abstracted by the first touch of the solvent water," and that soils retain salts in a condition of purely physical adsorption.

Stone and Monohan (98) noted that sterilizing loam increased the growth of soybeans 14 per cent, but that sterilizing in the same way decreased the growth of soybeans in subsoil 57.7 per cent. The subsoil pots showed poor, sickly development.

Schulze (91) noticed injurious effects from sterilization at 100 to 125°C. for 1 hour, in the early stages of growth, but later these plants became more vigorous and produced a larger crop, except peas and mustard on one soil.

Darbishire and Russell (16) heated soil at 90 to 95°C. and obtained very marked increases; the wheat yield (grain), from heated soil was 3.5 times as great as from unheated, and spinach, tomato and verbena, gave over four times the yield on the heated soil. The second crop, and even the third in one case, showed the same influence, though there was no increase in legumes. Heating to higher temperatures somewhat intensified the effect.

Koch and Lüken (55) heated a poor sandy soil in an autoclave for 2 hours under pressure of two atmospheres. This almost doubled the total soluble solids, quadrupled the soluble organic matter, but increased the soluble inorganic material only slightly. Even though heated and unheated soils were fertilized alike, the heated one produced the larger crop of oats. Injurious effects following heating were noticeable, but with crops seeded later in the season this influence was not great.

Rahn (81) made an extensive study of the effect of drying on soils. After drying at room temperature, he secured markedly increased bacterial activity, the difference being greater in heavy, rich soils, and increased growth of mustard.

Pickering (74) heated a soil at 200°C. for 2 hours, finding that 2 year-old apple trees made 63 per cent more growth, produced 48 per cent more leaves containing 52 per cent more dry matter than did similar trees in untreated soil. Heating in a moist condition increased the soluble matter, both organic and mineral, more than when heated in the dry condition. Heating soil at 100°C. in a closed vessel (75) increased total soluble matter 25 per cent in one case and 107 per cent in another, and in 11 soils an average increase from 0.052 to 0.360 per cent of soluble organic matter and from 0.111 to 0.475 per cent of total soluble matter was secured.

Gedroitz (25) found that sterilization brought about an increase in the solubility and assimilability of nutritive substances.

Russell and Hutchinson (86) report that heating soil at 98°C. increased the yield of rye 60 per cent and buckwheat 31 per cent.

Mann (64) gives a brief account of the "Rab" system of rice growing, viz., "burning a mass of branches of trees or cow dung on the land" where rice is to be seeded. He states that it is an almost universal practice on the trap and laterite soils of western India and is considered essential to rice culture there. The surface soil is heated sufficiently to change the bacterial flora, increase the soluble organic matter and improve the physical condition of the soil.

King (46) treated sand repeatedly with disulfonic acid to free it from all traces of nitric acid and organic matter, then charged it with potassium nitrate, which, after a time, was drained away. After the sand dried, 50 gm. were washed with 100 cc. of distilled water, the mass was stirred continuously for 3 minutes, the solution drained from the sand and the nitric acid determined. This was repeated until ten washings in all had been made. Table A gives the results of these determinations.

After the tenth washing the sand was treated with disulfonic acid, as is the residue in an ordinary nitrate determination, and was shown to contain 0.8 mgm. of nitrates, or nearly three times the amount recovered in the second washing, more than one-fourth as much as recovered in the first washing.

King reasoned from this that each sand grain "appropriated to itself" a film of water with potassium nitrate in solution and this film adhered to the particle so closely that in stirring after adding 100 cc. of distilled water the nitrate was given up by diffusion only and not by forming a mechanical mixture of the distilled water with the film.

Lyon and Bizzell (61) found that steaming for 2 to 4 hours under 2 atmospheres pressure increased water-soluble ammonia, organic nitrogen, nitrites and total soluble matter, but lessened the amount of nitrates. On standing 56 to 90 days after heating, there was a decrease in these soluble materials, except nitrates which remained constant. Wheat grown in

steamed soils at first showed injury, but later recovered and grew better than plants on the unsterilized soil.

Two soils heated for 2 hours at 2 atmospheres pressure and another for 4 hours (62), were extracted with 5 parts of water to 1 part of soil. Total solids and inorganic matter were increased from two to six times by this heating. All other constituents except nitrates were greatly increased by heating. No ammonia was found in any unheated soil and the concentration of nitrates was decreased by heating in every case. Wheat produced a much larger crop on the heated than on the unheated soil. and the same effect was very evident in the succeeding crop of millet, but wheat seedlings grown in a 1:1 extract made immediately after steaming, were affected unfavorably. Diluting the extract of steamed soil with distilled water, 3 to 1, improved the growth of seedlings but diluting the extract of untreated soil decreased the growth of seedlings.

The same writers (62) report that soils whose moisture content was maintained at about 25 per cent by adding distilled water, for periods of 56, 82 and 90 days after heating, steadily lost in total water-soluble material so that at the end of 90 days there was but slightly more

TABLE A[2]

Observed and computed concentration of nitrate in successive washings of sand in distilled water

NUMBER OF WASHING	WATER RETAINED IN SAND	CONCENTRATION OF SOLUTION	
		Observed	Computed
	gm.	*p. p. m.*	*p. p. m.*
1	12.7	35.750	43.4551000
2	13.2	3.300	4.8969000
3	13.1	0.451	0.5710100
4	13.4	0.174	0.0661390
5	13.05	0.138	0.0078153
6	13.3	0.128	0.0009023
7	13.5	0.110	0.0001059
8	13.5	0.110	0.0000126
9	13.5	0.110	0.0000015
10	13.4	0.110	0.0000002

than one-fourth as much soluble material present as immediately after heating. Nitrates decreased also, but in one soil they seemed to recover in part at the close of the period.

In a more extended experiment along the same line, soils stood 34 and 39 days after heating. Total solids, inorganic matter and ammonia nitrogen decreased rapidly, while there was a slight increase in nitrates in the same time.

Fletcher (23) relates that burning organic matter, twigs or manure, or both, greatly increased the yield of crops, but not to so great an extent as did heating the soil directly at 200 to 230°F.

Aitken (1) reports an instance of increased productiveness following heating of the surface garden soil by a "large and long-continued fire."

Howard and Howard (36) mention the beneficial effects, noted in parts of India, of exposing soil to the strong heat and light of the sun in April and May.

In soils kept moist for various lengths of time in open pans, Pickering (76) noted that the soluble matter, both organic and inorganic, increased as the temperature was raised from 30 to 150°C. The quantity of soluble matter decreased as the time since heating increased, up to 112 days when the last determination was made. In case of the inorganic matter at

[2] Letters used for tables derived from the literature.

100°C., however, there was an increase at both 44 and 112 days. When the soils were kept in sealed flasks there was an increase after 10 days in every case except that of inorganic matter at 125°C. When stored in sealed flasks for 43 days, the soils heated at 100 and 125°C. showed slight increase of soluble matter. When stored for 116 days there was a gain in the amount of soluble organic matter at the above temperatures but a loss in the quantity of soluble inorganic matter. Storing in light or darkness made no appreciable difference. In the case of the soil that had been heated at 125° there was no difference in organic matter whether stored at 15° or 5°, but that was a rather marked increase of inorganic matter for the lower temperature.

Pickering (77) secured increased growth of grasses and non-grasses (except in a preliminary experiment with the latter) when planted in soils previously heated. The amount of growth increased as higher temperatures were used. This increased growth correlates fairly closely with increase in soluble material, both organic and inorganic, resulting from heating.

Hall (26) says, in speaking of effect of sterilization of soil, "Approximately, the crop becomes doubled if the soil has been first heated to a temperature of 70 to 100°C. for 2 hours," while volatile antiseptics bring "about an increase of 30 per cent or more."

Russell (85) discusses briefly the beneficial effects of sterilization by heat and antiseptics, and assigns killing of the larger soil organisms which destroy the beneficial ones, as the explanation of the effect of this treatment.

Dyer (20) states that commercial vegetable growers near London partially sterilize their greenhouse soils with steam and if they use the ordinary amount of nitrogenous fertilizer the plants grow so rank as to "spoil their bearing capacity."

Seaver and Clark (92) heated soils from New York, Massachusetts and North Dakota. They found that the soluble matter in extracts of heated soils was generally six to ten times as great as that from the same soil not heated. The increase varied somewhat with the organic content of the soil, the temperature to which it was heated, and the period of heating.

Hinson and Jenkins (31) state that tobacco plants in steamed soils start quicker and grow faster than in unheated soil. They think this accelerated growth due to warming the soil, possible solvent action of the steam on the plant-food, but surely, in part, to change in the "microbe life in the soil."

In Kentucky, it is a common practice among tobacco growers to heat or burn the soil of the tobacco beds to kill weed seeds and disease.

Nagoaka (69) reports a material increase in solubility of phosphorus in dilute acids after heating the soil. He found autoclave heating had greater effect on solubility than other methods.

Peterson (72) heated wavellite and found that it increased the quantity of phosphorus soluble in 0.2 N nitric acid from 4.12 per cent of the total phosphorus, in unheated material, to 54.9 per cent in wavellite heated to 160°C., 49.0 per cent at 200°, and 98.7 per cent at 240°C. He noted little effect on soil at 100°C. but at 200°, after a five-hour treatment, there was a marked increase in solubility of phosphorus.

Ritter (83) experimented along the same lines as Rahn and found that drying increased bacterial activity, and with less effect on light than on heavy soils.

Fischer (22) comments on the work of Rahn and Ritter, but holds the chemical factor more important than the bacterial. He attaches much importance to oxidation since drying increases the amount of nitrates, even though it kills nitrifying organisms; also credits colloids and surface tension with playing important rôles.

Schreiner and Lathrop (90) in studying the chemistry of steam-heated soils made 1 to 4 extracts. Many organic compounds were found in heated soil that were not isolated from fresh soil. Dihydroxystearic acid was increased when present in the fresh soil and produced when not present. Seedlings were grown in the extracts for 10- and 15-day periods; extracts from heated soil depressed growths. Heating increased acidity.

Skalskii (94) found that sterilizing with chloroform and with heat increased the yield of crops by converting phosphoric acid and nitrogen into available forms.

Stone and associates (97) report greatly increased bacterial development in extracts from soils rich in organic matter, which had been heated previously, while it was retarded in extracts from poor soils. They consider the chemical factor most important in accounting for the effect of heating.

Leather (59) in studying the nitrate content of soils, at Pusa states that drying in the sun effected an increase as great as 400 per cent.

Seaver and Clark (93) found an increase in total soluble solids, organic matter, inorganic matter and total nitrogen, in soil when heated at 90°C. and still greater increases at 120, 150 and 170°C. Plant growth was accelerated by heating at 90 and 120°C. but retarded at the higher temperatures, which, however, increased the growth of fungi. They noted that heating increased acidity and suggest that this may account for the better growth of plants such as blueberry on "burned over" soils.

Lyon and Bizzell (63) have shown that when a soil has been heated to complete sterility by steaming and subsequently maintained at a moisture content of 25 per cent of its dry weight, the total solids decrease rapidly, as shown in table B.

TABLE B

Effect of standing on the water-soluble constituents of heated soils

	PARTS PER MILLION OF DRY SOIL		
	Total solids	Nitrates	Ammonia nitrogen
Soil 1, Dunkirk clay loam			
Freshly heated	3334	64.9	33.0
5 weeks after heating	2161	61.9	41.5
14 weeks after heating	1740	69.0	51.0
Soil 2, Volusia silt loam			
Freshly heated	3020	175.1	33.5
5 weeks after heating	2098	178.2	36.5
14 weeks after heating	1801	191.5	45.0
Soil 3, Dunkirk clay loam with extra organic matter			
Freshly heated	7194	234.0	84.1
5 weeks after heating	3288	306.0	79.5
10 weeks after heating	2719	282.5	96.0
19 week safter heating	2173	160.0	111.0

The nitrates have been affected but slightly, except in Dunkirk clay loam with extra organic matter, where there was an increase during first 5 weeks, but a rapid decline later, and, in general, an increase in ammonia nitrogen.

In another experiment, freshly heated soil had 1010 parts per million of total soluble solids and 246 parts per million of inorganic, while after 3 months the corresponding amounts are 590 parts per million of total soluble solids, and 126 parts per million of inorganic. When aerated during the 3-month period, there is a further decrease to 434 and 120 parts per million, respectively.

Russell and Petherbridge (87) state that plants grown on soils heated at 100°C., in comparison with unheated soils, have larger leaves of deeper green color and stouter stems, they flower earlier and more abundantly, the fruiting is more prolific, and they contain a higher percentage of nitrogen and sometimes phosphoric acid in their dry matter.

König, Hasenbäumer and Glenk (56) heated soil at 95°C. in a vacuum, which, in most cases contained markedly more water-soluble organic matter as well as total soluble solids

than did unheated soils. Heating at 150°C. still further increased the solubility of both organic and mineral matter. In most comparisons, heating increased the amount of water-soluble phosphorus, yet a few gave slightly less. Pot experiments with oats showed that heating in a vacuum at 95 to 98°C. increased the growth.

Wilson (106) secured slightly increased growth of plants in soil heated at 95°C., but retardation at higher temperatures, the effect varying with the kind of soil and nature of crop grown.

Buddin (8) found the nitrate content, immediately after drying in a thin layer in laboratory for 24 hours, unaffected, but reducing the moisture content further during 46 hours did increase the nitrate content slightly. When the dried soils were remoistened and incubated for 40 days, there was a marked increase in nitrates; untreated moist soil 36 parts per million, soil spread in gallery 46, and in glass-house 53.5 parts per million.

King (48) relates that the residents of northern China build flues ("Kangs") of sun-dried bricks made of "soil or subsoil mixed with short straw or chaff." After two to four years' use, these flues become defective, so that they must be replaced. When removed the bricks are finely pulverized and used as fertilizer, being planted in hills with the seed. The soil while used as a flue has become thoroughly air-dry and on the inside of the flue, undoubtedly, much of it has at times been freed of uncombined water. During this long-continued drying, the plant nutrients have been rendered more available and this fact is made use of by the Chinese farmer. King (47) suggests "absorption of the products of combustion" by the "brick" as an additional factor in giving them value as fertilizer.

Kelley and McGeorge (40) review briefly the history of burning soils. There are, in Hawaii, large areas of heavy soil which do not, when first plowed, produce satisfactory crops. It requires several months of cultivation before crops thrive. It has been noticed that on small spots where brush has been burned cotton grows exceptionally well. It is suggested that this effect may be due to heating the soil rather than to the soluble oxides of phosphorus, potassium, calcium and magnesium in the ash, since fertilizers do not produce such beneficial results.

They report results of analyses of the 1:5 water-extract of a brown ferruginous clay soil and its subsoil, and a similar type which had been plowed and was growing pineapples. Determinations were made on fresh, air-dried and oven-dried samples, extracted respectively for 1 hour, 24 hours and 7 days. Phosphoric acid (P_2O_5) was always highest in the oven-dried soil, manganese oxide (Mn_3O_4) always higher in air-dried than in fresh soil (not determined in oven-dried soil). Lime (CaO) was highest in the oven-dried soil in three of nine comparisons only. Magnesia (MgO) varied, in some samples higher in the air-dried. Sulfuric acid (SO_3) was highest in the fresh soil oftener than in either of the others, and potash was highest in the air-dried soil (with one exception) in the two surface soils, while in the subsoil the fresh soil held first place.

When all the comparable data are considered we see that in three cases the fresh soil was highest in total soluble solids, in three cases the air-dried and in the other three cases the oven-dried soil stood first. So no conclusion as to the effect of heating on total soluble salts can be drawn from these figures.

In general, extracting for 24 hours or for 7 days gave but slightly higher results than extracting for 1 hour, except in the case of phosphorus which increased in solubility with longer extraction. In two out of eight trials, heating at 100°C. increased the nitrate content; in four soils it was decreased while in the other two there was no change. As the soil was raised to higher temperatures, 150 to 200 and 250 nitrates decreased rapidly until almost none was recovered at the highest temperature.

These investigators think both chemical and physical factors enter into an explanation of the effect of drying on the soluble constituents of soils, but that "the most important set of factors affecting the solubility of inorganic soil constituents are physical in nature. Also that the physical factors act through the effect of changes in soil moisture on the physical properties of the soil." "The conditions conducive to the formation of a colloidal state and

the subsequent relation of heat to the destruction of this colloid are two of the most important of these factors." When soil contains some capillary or film water this moisture is distributed about the particles as a thin film varying in thickness with the quantity of water present in any given soil. It is stated that the moisture film in air-dry soils is held with a force equal to 10,000 atmospheres and that under such conditions "the concentration of film water with reference to the mineral matter should be much greater than that of the free or capillary water in the soil." They hold that air-dried soils should, and their results are claimed to, show least solubility.

The films with organic and inorganic matter in solution may be looked upon as colloidal in nature. Upon heating to 100°C. alteration in the film occurs through evaporation and by partial dehydration of the colloids, destroying the pressure by which the film was previously held around the particles. During evaporation the concentration of the soil moisture would increase to the saturation point, after which mineral matter would be deposited with further evaporation.

The solution obtained upon adding water to oven-dried soil should be of greater concentration than that from air-dried soil. With water films absent and the colloids altered, the water has more ready access to the soil particles. They found some mineral constituents more soluble at 250°C. than at 100°C. and think it due to "more complete elimination of soil moisture and especially the water of chemical combination."

Hulett and Allen (37) showed that the concentration of the solution in equilibrium with a curved surface is greater than that in equilibrium with a plane surface and that gypsum is most soluble in water at 40°C. Above 80°C. it is less soluble than at 0°C.

McGeorge (66) reports further results of heating soils in sunlight, in an oven for 2 hours at 80, 110 and 165°C., and in an autoclave for 1 hour at 10 pounds pressure. Onions and cowpeas showed detrimental effects while millet showed increased vigor with the higher temperature of sterilization. Heating gave better results than volatile antiseptics.

Ehrenberg (21) speaks of the old custom of using as fertilizer old garden walls made of soil and says that many soil workers have noted an improvement as the result of a soil drying out. He thinks soils rich in organic matter, only, are affected materially by drying.

Hall (27) allowed eight pots of similarly treated Dunkirk clay loam to dry from October 19 to March 1. At this time the moisture content of four pots was brought up to 20 per cent and held there until April 12. The other four continued to dry until March 19, at which time the moisture content was 1.8 per cent. The first four pots had an average of 847 parts per million (of dry soil) of total soluble solids and 5.18 parts per million of nitrates, while the average for the four air-dry soils was 1303 parts per million of total salts and 324 parts per million of nitrates, a marked increase due to drying. In October, a sample of the soil was dried. In March it had 1628 parts per million of total soluble-matter and 397 parts per million of nitrates. A sample of the original soil bottled at 12.2 per cent moisture in October had in March 1459 parts per million total salts and 495 parts per million of nitrates. This shows that nitrification had been active as in the soil which dried to March 1.

Klein (53) conducted experiments with Dunkirk clay loam (*a*) low in organic matter and (*b*) well supplied with organic matter, this being timothy sod which had been piled up and allowed to decay. Keeping soil *a* at 15, 20, 25 and 30 per cent moisture and *b* at these moisture contents with an additional sample at 40 per cent, gave an increase in growth of wheat on *a* with a decrease in moisture, and the same general relation held for *b* except that the soil with 40 per cent moisture gave practically the same yield as that with 15 per cent. There is no important difference in the yield of buckwheat following the wheat. Soil *a*, unplanted, contained more total soluble solids with the lower moisture contents, while soil *b* showed an increase in total soluble solids with an increase in the water content. Nitrates decreased with the lowering of the water content. Difference in water content had no effect on solubility of potassium, calcium and phosphorus in this soil. Air-drying reduced the nitrates, but when later brought up to and kept at optimum moisture content for various periods greater than 16 days, the nitrates increased materially. The nitrifying power and power to produce carbon dioxide is, in general, affected in the same way.

Wilson (105) found that heating at 60 to 150°C. for 2 hours increased the amount of soluble matter and changed the physical condition so that its water-holding capacity was affected. He accounts for increased productivity on these grounds.

Buck (7) reports results of a study on the effect of heat on soils, by Mann who found the water-soluble constituents increased with the rise in temperature to which the soil was heated. He notes greater growth of rice seedlings immediately after heating, quite the reverse of his experience with other plants, which may be due to the ability of the rice seedling to withstand any harmful effects of, or to use in growth, the ammonia which many hold to be a result of heating.

The work of Kelley and Thompson (41) shows that nitrates undergo decomposition, gradually disappearing as the temperature is raised. Only slight decomposition took place at 100°C. Steam heating at 2 atmospheres produced effects similar to those resulting from heating at 150°C. without pressure.

Bouyoucos (5) heated sandy loam, loam, clay and peat at 15 atmospheres pressure in an autoclave for three hours, thereby increasing the water-soluble material, as shown by the depression of the freezing point, respectively, 75, 50, 190 and 333 per cent.

In explanation of the effect of heat, he points out that water films in intimate contact with the soil particles are more concentrated than capillary or interstitial water, due to the slowness of diffusion. If only the capillary water is extracted, the quantity of soluble matter recovered would be less than the total actually in solution in the soil moisture. He suggests that adsorption may account for a higher concentration at the immediate surface of the particles than in the bulk of the solution. Furthermore, there is wide variation in the solubility of the minerals composing the soil and because of the extremely slow rate of diffusion, different mineral particles would be enveloped by films of varied concentration. This, too, would interfere with recovering from moist soil all of the soluble material.

Allen and Bonazzi (2) quote Stevens and Withers showing "only about 40 per cent of the nitrates were recovered by 1:3 extraction when small quantities were added and more than twice this amount when larger quantities were added." Allen and Bonazzi recovered in the first extraction (1:5 with 100 gm. of soil) from 65.9 to 83.9 per cent of the nitrate added, or as an average of ten results reported, 77.4 per cent.

Potter and Snyder (78) report a recovery of 93 to 97 per cent of the nitrate added at the rate of 3 parts per million of soil (phenoldisulfonic acid method).

These authors (79) report complete extraction of nitrates when 1 part of soil is shaken with 2 parts of water for 30 minutes (aluminum reduction method).

Johnson (38) reports preliminary results, showing that heating increased the solubility of minerals and the growth of plants. Heating to 250°C. produced more water-extractable substance than lower temperatures.

Skalskij (95) in studying methods of sterilization heated soil in an autoclave for 1 hour at 2.5 atmospheres. Plants in this soil grew as well as those in soil receiving complete fertilization, the number of bacteria was greater than in the untreated soil, the inoculation coming from the air. The improved fertility was due to a large increase in the soluble phosphorus, from 47 to 121 per cent, and while the soluble nitrogen content was not affected by heating, the dark green color clearly showed an increase in the available nitrogen.

Connor (15) reports a reduction in acidity as a result of heating.

Coleman, Lint and Kopeloff (14) found that the soluble solids recovered by 1:4 extraction of a moist Penn clay loam soil (25 per cent water on the dry basis) after intermittent partial sterilization at 82°C. for 1 hour on each of 5 consecutive days, was increased 46 per cent, but the amount of soluble salts was not appreciably increased after the first day's heating. The air-dry soil (4.5 per cent moisture) when similarly treated showed no increase in water-soluble solids. Sterilization by moist heat at 120°C. for 15 minutes at 15 pounds pressure increased the water-soluble solids recovered 0.0220 to 0.1805 gm., an amount 8.2 times as great as that recovered from the original soil. It should be noted also that volatile antiseptics applied as vapor in a partial vacuum, increased the water-soluble solids in air-dry soil 22 per

cent and 25 per cent in the moist soil. When the volatile antiseptics are applied with heat (82°C.) and pressure, the amount of soluble solids in moist soil is increased 25 per cent.

Christensen (10) noted that air-dry soil had its power to liberate acid from calcium acetate considerably increased as compared with that of fresh moist soil.

Koch (54) determined the effect of sterilization on the concentration of the soil solution by means of the freezing-point method. Concentration was increased more in the heavy soils. Steaming was more effective than sterilizing with formalin. Using formalin 1:50 and steaming at 10 pounds pressure increased the concentration of the soil solution more than any other method used, in fact to three times the original concentration. In Sassafras and Penn loams it was increased, respectively, 0.24 and 0.3 atmospheres. Sterilization by the so-called "commercial" methods increased the concentration of the soil solution, varying with soil and method of sterilization. Heating with steam at 10 pounds pressure for 1 hour increased the concentration of the soil solution 0.56 atmosphere in a loam soil; with a Norfolk sand the increase was but one-fourth as great.

Stewart (96) studied water extracts of thirteen soils, of two distinct types both planted and fallow, finding as did King (44) that "poor" soils yielded extracts containing solids less soluble than solids in extracts from "rich" soils.

Hartwell and Pember (28) while investigating the effect of aluminum on barley and rye, compared unheated acid soil with samples heated at 100, 260, 360, and 420°C. The lime requirement was markedly reduced by heating and the reduction increased somewhat with the temperature. The weight of green tops of rye was reduced by heating except that heating at 420°C. caused no difference in yield. The yield of green barley tops was decreased at both 100 and 260°C. but increased at both of the higher temperatures.

Potter and Snyder (80) state that "the amount of ammonia was increased by all the heat treatments, the higher temperatures to which the soils were heated giving in general greater increases;" also that dry heating at 100°C. did not materially affect nitrates, but at 10 pounds pressure in an autoclave for 9 hours, nitrates were markedly increased while a temperature of 200°C. caused an almost total disappearance of nitrates.

Johnson (39) heated soil at 250°C. The yield of tobacco was increased 571 per cent on muck, 473 per cent on Waukesha silt loam, 150 per cent on clay, 96 per cent on fine sandy loam and 62 per cent on virgin sandy loam. A single heating gave a larger yield than did heating two to eight times at 115°C. He found, also, enormous increases in concentration of the soil extract, as shown by freezing-point determinations. Heating at 250°C. caused the highest concentration.

He classifies under eight heads the published theories explaining the effect of sterilizing soils on plant growth, three of which have some bearing on the problem in hand; (*a*) "Modified organic compounds" as already mentioned from Schreiner and Lathrop (90). (*b*) "Modified inorganic soil compounds." This theory is supported by many investigations which show that an increase in inorganic plant nutrients occurs on heating soils. (*c*) "Physical theories." The author says the physical "theories are not subscribed to by any author in particular at the present time, although it was quite generally believed at one time that all the benefit derived from burning the soil was due to purely physical changes. Some of the physical factors which play a part in soil fertility are, however, coming to be regarded as very influential in conjunction with chemical factors."

Beaumont (3) showed that drying soil caused a decrease in the amount going into suspension in distilled water or 4 per cent ammonia. Oven-drying soils and then putting them under water logged conditions increased the quantity of iron compounds soluble in dilute hydrochloric acid. He states, also, that "sterilization checked the formation of this easily soluble colloidal material."

Noyes (70) while working with adsorption of different radicals by soils and decaying leaves detected no adsorption of nitrates. He holds "nitrates are completely recovered from soil in one extraction by water, and nitrates added to soil are completely recovered in addition to those present in the soil." He noted also that the lime requirement of a residual limestone soil was higher when not heated than when evaporation is carried on in the usual way.

Robinson (84) states that the lime requirement of soils, as shown by the Veitch method, is affected by (*a*) "the temperature at which evaporation is made," (*b*) continued heating after soil is dehydrated, (*c*) length of time during which treated and dried soil is in contact with water, and (*d*) the source of heat, such as steam or sand bath or hot plate.

An enormous amount of interesting work on the soluble-solid content of soils under many different conditions of cropping is reported by King (42, 43, 44, 46), King and Jeffery (49), King and Whitson (50, 51, 52) and Whitson (104).

The literature of drying, heating and sterilizing soil has been quite extensively reviewed by Lyon and Bizzell (62) and (63), Schreiner and Lathrop (90), Kelley and McGeorge (40), Klein (53), Hall (27), Kopeloff and Coleman (57), Stewart (96), Beaumont (3) and Johnson (39).

SUMMARY OF LITERATURE

1. Heating soil in various ways for its beneficial effect on crops is an ancient practice.

2. For several decades past, commercial greenhouse men have sterilized the soil used, to kill detrimental organisms, and have noted beneficial results other than from sterilization, particularly increased growth of leaves and stems.

3. Much careful experimental work on heating and drying soils has been reported, both before soil organisms were recognized, and in connection with soil-biology studies, which shows that drying and heating soil at 100°C., or higher, increases its productiveness, even though germination may be retarded and early growth depressed.

4. The literature shows that the quantity of soluble mineral and organic constituents recovered by extraction with distilled water is increased by heating. The increase bears some relationship to the temperature of heating, the maximum of soluble constituents being found at about 250°C., above which the total salts recovered decreases.

5. Investigators are not in general agreement as to the effect on nitrates of heating at 100°C. Many workers note a decrease as the temperature is further raised and almost total disappearance of nitrates at 250°C.

6. Soil workers are not a unit as to the cause of the increase in soluble material due to drying and heating. Some hold the effect of heating to be largely physical; others that it is mainly chemical, and still others lay most stress on the biological phase. Nearly all admit that the physical is usually a factor and others add colloids as a physio-chemical factor.

7. There is wide variance of opinion as to the degree to which nitrates are recovered by one or more extractions.

EXPERIMENTAL WORK

Introduction

While the literature shows that an enormous amount of work has been done on the effect of heating at a wide range of temperatures and under varied moisture contents on the amount of soluble constituents of soils, it was considered desirable to study the effect of drying at 105°C. for 8 hours, the ordinary method of driving of so-called hygroscopic moisture, on the total soluble solids that may be recovered by 1:5 extraction with distilled water.

Method of collecting soil

The surface vegetation was removed and the surface of the soil leveled. Steel tubes of 2½ inches inside diameter at the cutting edge and 2⅝ inches above it were used. A block of wood was placed on the tube which was then driven into the soil with a sledge hammer to a depth of 8 inches. The soil within the tube constituted the "tube" surface sample. Heavy paper held in place by rubber bands was immediately placed over each end of the tube to reduce evaporation and aeration.

A sample was then collected from immediately around the tube with a 1½-inch auger to the same depth. This was immediately placed in a 2-quart Mason jar with a minimum of evaporation and aeration. This was the "auger" surface sample.

The hole was enlarged and dug out with a spade to a depth of 12 inches and the soil from 8 to 12 inches discarded. The tube was driven down from 12 to 20 inches and protected as before. This stratum 12 to 20 inches constitutes the "tube" subsurface sample. The "auger" sample was collected and sealed as before. All samples were taken to the laboratory. The paper cap at the top of the steel tubes was removed and melted paraffin poured on so as to seal it. When the paraffin had solidified, the tubes were inverted, a portion of the soil removed and the tube sealed as above. Both tubes and jars were properly labeled and placed in a refrigerator, at a temperature of 8 to 12°C. in order to keep the soil as nearly in its original condition as possible by minimizing biological activity.

All soil samples were collected and treated in this way.

General procedure

Duplicate 100-gm. samples were weighed into aluminum dishes of known weight and placed in an electric oven at a temperature of about 105°C. for 8 hours to determine the total moisture. After these had been weighed, water-free soil from another dish was added to each to make exactly 100 gm. of water-free soil for the determination of total soluble salts and nitrates.

Lyon and Bizzell (63) have shown that aerating after heating has a marked influence on the disappearance of total soluble solids. For that reason, all soils both before and after heating were kept in closed containers to reduce aeration to a minimum.

The percentage of moisture in the fresh soil was calculated on the basis of water-free soil as 100 per cent. The method of obtaining a quantity of moist soil precisely equivalent, to 100 gm. of water-free soil, was to add to 100 gm. of moist soil 1 gm. of moist soil for every per centum of moisture in it. King's (45) method was followed throughout. Five hundred cc. of distilled water was used with 100 gm. of water-free soil. The soil was placed in a mortar, sufficient water added to make a thin paste, rubbed with a pestle for 3 minutes, and the mixture transferred to a porcelain pitcher, stirred a moment, and allowed to stand 20 minutes. Soil and water were then transferred to a Pasteur-Chamberland filter and a pressure of 15 to 20 pounds applied.

In the case of moist soil containing, for example, 20 per cent water, 480 cc. of water was added, making a total of 500 cc. precisely the same as in case of the dry soil. In this way, the calculation is simplified and the comparison is more accurate than where 100 gm. of both moist and water-free soil are used and 500 cc. of water added to each. For example, if 100 gm. soil contains 20 per cent water, only 80 gm. of soil are washed, 500 cc. + 20 gm., or cc., of water from the soil, a total of 520 cc. The ratio of soil to water is 1:6.5, instead of 1:5, as with 100 gm. of dry soil to 500 cc. of water, for which our plan calls.

The first 50 cc. of the soil extract was discarded. Two 125-cc. portions from each of the duplicate soil samples, or four portions from each soil, were placed in silica dishes and reduced to dryness on a water-bath, dried in the electric oven at 105°C., cooled in a desiccator, and weighed on an analytical balance. This weight of solids represents one-fourth of what was actually dissolved in the extract from the original 100 gm. of soil.

For determining nitrates, two 50-cc. samples from each soil duplicate, four in all from each soil, were evaporated to dryness, after adding a few drops of a saturated solution of sodium carbonate, and nitrates determined colorimetrically according to Schreiner and Failyer (89).

Experiment 1

The object of this experiment is to study the effect of oven-drying on the soluble solids and nitrate content of four types of soil found in this locality and which cover a wide range of physical composition.

Samples of four soils were collected October 24, 1918, from the Station farm at Ithaca.

1. *Dunkirk silt loam*
   A. Surface, yellow, heavy silt to clay loam
   B. Subsurface, yellow silt loam

Both surface and subsurface soil contained a few small pebbles and were very low in organic matter. This soil had been growing a heavy sod of Kentucky bluegrass for the past ten years.

2. *Genesee gravelly loam*
   A. Surface, brown gravelly loam, much coarse material
   B. Subsurface, yellowish brown gravelly loam to gravel

This was a very coarse gravelly loam containing only a small percentage of sand, little more than a trace of silt and clay, and was low in organic matter. It had been growing alfalfa and some Kentucky bluegrass for several years.

3 *Dunkirk fine sandy loam*
   A. Surface, yellow fine, sandy loam
   B. Subsurface, yellow sandy loam, sand below 18 inches

The first 18 inches of this soil were very uniform in texture. The land was naturally not well supplied with organic matter, but had been manured somewhat in recent years. It had been fallowed during 1918 and was being planted to young orchard.

4. *Volusia stony loam*
   A. Surface, brown stony loam, loamy phase, not many stones in sample
   B. Subsurface, yellowish brown stony loam, more fragments of stone than in surface.

This sample was taken near the boundary between this type and Volusia silt loam as mapped by Bonsteel, Fippin and others (4). This soil has been growing mixed grasses for some time.

Table 1 gives the results of the determinations of total soluble solids and nitrates in heated and unheated soils. The figures in the last column of tables 1, 2, 3 and 6 are in each case the product of the probable error of the difference and 3.81. It is the quantity of total solids in milligrams that the dry soil must exceed the moist (or air-dry) in order that the variation may be considered significant, that is, that the odds be 30:1 in favor of the difference being due to variation in the conditions of the experiment and not to error in manipulation.

From table 1, it is readily seen that in every case the dry soil yielded more total soluble solids than did the moist soil. The amount of nitrates varies considerably. In five of the eight tube samples, denitrification had reduced the nitrates to zero, of the remaining eleven comparisons, the moist soil was slightly higher in nitrates in six, while the dry was ahead in five cases, so no conclusion as to effect on nitrates can be drawn from this experiment. It

should be stated that there was considerable variation in the period between sampling and the determination (the last one being made August 15, 1919) so that biological action may account in part for the wide variations and the frequent high probable error of the difference. Occasionally the ice was allowed to get low in the refrigerator so the temperature became high enough for good bacterial growth. There is, however, no case in which, if the nitrates are calculated as $Ca(NO_3)_2$ and deducted from the total solids, that the conclusion or its significance is altered.

Experiment 2

The purpose of this experiment was to study the effect of storage for nine weeks at 8 to 12°C. on the quantity of soluble material when the soils were kept under somewhat carefully controlled conditions of moisture content, but under different conditions of aeration. Determinations of total soluble solids were made on both moist and dry soils.

On October 23, 1919, three soils, Dunkirk silt loam (sod), Dunkirk silt loam (stony), and Dunkirk sandy loam, were collected and treated as noted above. The first and third are the same soils as were used in experiment 1 and are similar to them in every way, these samples being taken within a few feet of the others. The third soil, Dunkirk silt loam (stony), was taken from a field growing rye and vetch. It had been well manured before seeding and has received considerable fertilizer during the past few years.

Procedure

The tube samples were sealed with paraffin at once and kept in the refrigerator for a period of 68 days to determine the effect of this method of storage. The jars containing the auger samples were kept in the refrigerator also. Total soluble solids and nitrates were determined on all of the auger samples within the next 5 days, beginning on October 24.

The remainder of each of these samples was kept in the ice-box in an open jar to allow free aeration, weighed at intervals of 1 week, and distilled water added to make up for any loss by evaporation in order to detemine the effect of this method of storage for 65 days. The principal difference in the two methods of storage is in the opportunity for aeration in the auger sample in open jars, particularly since the water evaporating was compensated at intervals of one week.

The results of the determinations are stated in table 2.

These figures show that the oven-dried soil compared with the original moist soil had in the two silt loams three to five times as much soluble solids and in the sandy loam twice as much.

The Dunkirk silt loam (sod) contained very little soluble matter, either mineral or organic, and but little more than a trace of nitrates, 1.7 parts per million in the fresh moist sample. This is in close accord with determinations made on this soil collected the previous season, though both nitrates and total solids were somewhat higher then.

TABLE 1

Soluble material obtained by 1:5 water extraction

METHOD OF SAMPLING	STRATUM	MOISTURE CONTENT†	NITRATE	TOTAL SOLUBLE SALTS				MEAN	E‡	E × 3.81
				I		II				
				A	B	A	B			
Dunkirk silt loam										
		per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*			
Auger.....	Surface	22.76	0.75	34.5	36.0	35.2	35.2	35.2±0.183*	0.6837	2.605
		Dry	0.61	56.0	52.2	56.0	55.2	54.8±0.6588		
Tube.....	Surface	22.01	None	50.0	48.8	49.6	49.6	49.5±0.1708	1.347	5.132
		Dry	None	74.4	75.2	68.8	69.6	72.0±1.3364		
Auger.....	Subsurface	21.53	1.48	16.8	13.6	15.2	14.4	15.0±0.3660	0.4621	1.761
		Dry	2.34	43.2	42.4	41.0	42.4	42.2±0.3173		
Tube.....	Subsurface	21.80	2.12	17.6	18.4	17.2	16.8	17.5±0.2439	0.5453	2.078
		Dry	2.09	44.8	44.0	42.4	42.4	43.4±0.4878		
Genesee gravelly loam										
Auger.....	Surface	27.87	33.52	87.2	85.6	87.2	88.8	87.2±0.3904	4.458	16.98
		Dry	36.92	242.0	233.4	222.8	220.8	231.0±4.4412		
Tube.....	Surface	28.46	None	111.2	112.8	96.0	96.0	104.0±3.9041	4.365	16.63
		Dry	None	152.8	160.0	148.4	148.4	152.4±1.9522		
Auger.....	Subsurface	13.84	2.60	20.0	18.4	19.6	20.0	19.5±0.2688	0.3321	1.265
		Dry	1.61	38.4	39.2	37.6	38.4	38.4±0.1952		
Tube.....	Subsurface	11.51	None	14.4	15.2	9.6	8.0	11.8±1.3421	1.350	5.143
		Dry	None	20.8	20.0	20.0	20.0	20.2±0.1464		

Dunkirk sandy loam										
Auger.....	Surface	17.14	18.70	56.8	56.0	56.8	58.0	56.9±0.2684	1.297	4.942
		Dry	19.38	144.8	146.0	140.4	140.0	142.8±1.269		
Tube.....	Surface	17.37	10.81	34.0	33.6	33.6	32.8	33.5±0.1708	0.2978	1.135
		Dry	9.18	78.4	78.4	79.2	79.6	78.9±0.2440		
Auger.....	Subsurface	18.26	9.85	32.8	32.0	31.2	31.2	31.8±0.3172	0.7533	2.870
		Dry	7.31	87.2	87.2	88.0	91.2	88.4±0.6832		
Tube.....	Subsurface	24.13	None	6.4	6.4	7.2	7.2	6.8±0.1952	0.2754	1.049
		Dry	None	12.8	13.6	13.6	14.4	13.6±0.1952		
Volusia stony loam										
Auger.....	Surface	29.00	14.54	49.6	50.0	48.0	46.4	48.5±0.6345	3.092	11.78
		Dry	13.90	140.0	140.8	152.8	152.8	146.6±3.0258		
Tube.....	Surface	27.76	1.10	11.2	12.0	13.6	13.6	12.6±0.4881	0.8397	3.199
		Dry	0.94	107.2	106.4	108.8	110.4	108.2±0.6833		
Auger.....	Subsurface	18.93	12.1	15.2	14.4	16.0	16.8	15.6±0.3904	0.5875	2.239
		Dry	12.5	42.4	39.2	40.8	40.4	40.7±0.4392		
Tube.....	Subsurface	19.42	None	9.6	6.4	7.2	9.6	8.4±0.5856	0.6175	2.353
		Dry	None	35.2	34.4	33.6	34.4	34.4±0.1952		

* The probable error of the mean R was calculated by Peter's approximation formula, given by Mellor (67).

$$R = \pm 0.8453 \frac{\Sigma (+v)}{n\sqrt{n-1}}$$

R is the probable error of the arithmetical mean of all determinations; $\Sigma (+ v)$ is the sum of the deviations of every determination from the mean, their sign being disregarded; n is the number of determinations actually made.

† All moisture percentages in this paper are based on weight of soil, water-free after drying in the oven at 105°C. for eight hours.

‡ The probable error of the difference (E) is calculated by means of the formula $E = \sqrt{E_1^2 + E_2^2}$ where E_1 and E_2 represent the probable error of the means.

TABLE 2

Effect of method of storage on soluble material in soils

STRATUM	KIND OF SAMPLE	MOISTURE CONTENT	NITRATES	TOTAL SOLUBLE SATS				MEAN	E	E × 3.81
				I		II				
				A	B	A	B			
Dunkirk silt loam (sod)										
		per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*			
Surface	Auger, fresh	20.50	0.17	15.2	13.6	16.0	14.4	14.8±0.3904	0.6249	2.381
		Dry	0.52	72.0	71.2	74.4	72.8	72.6±0.4880		
Surface	Auger, stored	19.42	1.54	13.0	12.0	12.8	12.0	12.4±0.2196	0.4061	1.547
		Dry	1.28	62.4	62.4	60.8	63.2	62.2±0.3416		
Surface	Tube, stored	19.93	0.24	10.4	10.4	12.0	10.4	10.8±0.2928	0.6987	2.662
		Dry	None	47.2	47.2	50.0	50.0	48.6±0.6344		
Subsurface	Auger, fresh	15.83	None	7.2	6.4	7.2	6.4	6.8±0.1952	0.3518	1.340
		Dry	None	20.0	19.2	19.2	20.8	19.8±0.2928		
Subsurface	Auger, stored	14.62	0.39	8.0	8.4	8.0	8.0	8.1±0.0976	0.2182	0.831
		Dry	0.30	28.8	28.0	29.6	28.8	28.8±0.1952		
Subsurface	Tube, stored	15.43	None	11.2	9.6	9.6	8.8	9.8±0.3416	0.4499	1.714
		Dry	None	22.4	21.6	21.6	23.2	22.2±0.2928		
Dunkirk silt loam (stony)										
Surface	Auger, fresh	21.43	7.30	40.0	39.2	38.4	40.0	39.4±0.2928	0.4499	1.714
		Dry	5.61	144.0	144.0	143.2	145.6	144.2±0.3416		
Surface	Auger, stored	20.04	10.30	44.8	46.4	46.4	46.4	46.0±0.2928	0.5278	2.011
		Dry	9.95	125.2	126.4	126.4	124.0	125.5±0.4392		

Surface	Tube, stored	19.67	1.77	40.0	39.2	39.6	39.2	39.5±0.1464	0.2440	0.930
		Dry	1.32	130.4	131.2	131.2	130.4	130.8±0.1952		
Subsurface	Auger, fresh	13.62	3.64	26.4	25.6	26.4	26.4	26.2±0.1464	0.4169	1.588
		Dry	2.61	85.6	84.0	83.2	83.2	84.0±0.3904		
Subsurface	Auger, stored	12.43	3.67	28.0	27.2	29.6	28.8	28.4±0.3904	0.6249	2.381
		Dry	3.69	81.6	78.4	80.0	79.2	79.8±0.4880		
Subsurface	Tube, stored	13.68	1.32	23.2	20.8	20.8	21.6	21.6±0.3904	0.6249	2.381
		Dry	1.33	84.8	84.8	83.2	82.4	83.8±0.4880		
Dunkirk sandy loam										
Surface	Auger, fresh	17.95	10.65	36.8	36.8	37.6	36.8	37.0±0.1464	0.6036	2.300
		Dry	8.65	86.4	85.6	82.4	84.8	84.8±0.5856		
Surface	Auger, stored	16.56	11.62	35.2	34.4	32.8	35.2	34.4±0.3904	0.6249	2.381
		Dry	11.60	68.8	67.2	70.4	69.6	69.0±0.4880		
Surface	Tube, stored	17.22	20.00	46.4	47.2	47.2	46.4	46.8±0.1952	0.2440	0.930
		Dry	17.30	75.2	76.0	75.2	75.2	75.4±0.1464		
Subsurface	Auger, fresh	14.22	1.00	11.2	10.4	11.2	10.8	10.9±0.1464	0.4629	1.764
		Dry	0.64	20.8	20.8	18.4	20.8	20.2±0.4392		
Subsurface	Auger, stored	12.76	0.79	9.2	10.4	9.6	9.6	9.7±0.1708	0.3389	1.291
		Dry	0.62	16.8	16.8	18.4	17.6	17.4±0.2928		
Subsurface	Tube, stored	13.45	0.33	10.0	10.0	9.6	8.8	9.6±0.1952	0.2440	0.930
		Dry	0.26	19.2	18.4	19.2	19.2	19.0±0.1464		

Close scrutiny of this table reveals that in three cases the stored auger sample is higher in total soluble solids and in three cases, lower than the fresh sample. Determinations made on fresh auger samples and on the stored sealed-tube samples, therefore, are comparable.

In each soil there is evidence of nitrification in the open-jar-auger sample, the increases in the three soils being in order from 1.7 to 15.4 parts per million from 73 to 103 and from 106 to 116. In some cases the nitrates run higher in the sealed-tube soil and in some lower than in the fresh soil, so from these meagre data no conclusion can be drawn as to the effect on nitrates of storing in sealed tubes for a period of 68 days.

It appears that the effect of storing at a temperature of 8 to 12°C. in open jars for 68 days with the restoration of moisture evaporated at intervals of a week or in sealed tubes for 65 days does not materially affect the quantity of total soluble solids as determined by 1:5 extraction with distilled water, all of the soils being in the moist state. Storing decreased the soluble solids as determined in the water-free condition in five of the six comparisons made, the losses, however, were not great.

Experiment 3

The object of this experiment was to study the effect on soluble solids and nitrates of sun-, air- and oven-drying on soils that had been water-logged for a considerable period, if, indeed, ever dry since their formation.

In July, 1919, samples of a drab silt loam were collected near Cayuga Lake close to the west wall of the valley at the mouth of a small tributary. The surface and subsurface were taken with a steel tube, described above. The subsoil, 20 to 40 inches, was taken with an auger in the bottom of holes left by the tube after taking subsurface stratum. The soil suffered little, if any, change due to aeration and since it was very wet, containing 73 to 108 per cent of moisture, and the glass containers were sealed as soon as possible and placed in the ice-box on reaching the laboratory.

Determinations were made the same as in experiment 1 during the next few days on the wet and corresponding oven-dry samples and in the same way on the sun-dried samples on August 8.

Another set of samples was collected on October 16, 1919, south of Ithaca, near the east wall of the valley at the mouth of a small stream. This soil, also a drab silt loam, was not so wet as the other and contained nitrates in the subsurface, whereas the other soil had none in the subsurface.

In every case the wet soil was mixed thoroughly by hand and the total moisture determined in triplicate instead of in duplicate, as with all other soils.

The "wet" samples were oven-dried during the night and determinations made the following day, October 17. Half of the soil was set out to dry in the laboratory, as there is usually not much sunshine and rainfall is frequent here at this season. Determinations were made as soon as the soil was entirely air-dry.

The results are given in table 3.

TABLE 3

TABLE 3

Effect of drying on soluble material in swamp soils (drab silt loam)

CONDITION	STRATUM	MOISTURE CONTENT	NITRATES	TOTAL SALTS RECOVERED				MEAN	E	E × 3.81
				I		II				
				A	B	A	B			
Inlet Valley, near Cayuga Lake and west valley wall										
		per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*			
Wet as it came from field	Surface	73.67	3.66	101.6	102.4	97.6	96.8	99.6±1.1712	1.207	4.599
		Dry	3.06	161.6	160.0	161.6	160.8	161.0±0.2928		
	Subsurface	83.65	None	91.2	88.0	82.0	83.2	86.1±1.7081	2.071	7.814
		Dry	None	137.6	136.8	140.8	143.2	139.6±1.1712		
	Subsoil	107.85	None	63.2	64.0	64.8	62.8	63.7±0.3416	2.7542	10.492
		Dry	None	199.2	200.0	185.6	191.2	194.0±2.7328		
Air dried in the sun	Surface	1.88	4.60	122.4	121.6	121.6	124.0	122.4±0.3904	1.6093	6.131
		Dry	4.60	185.6	177.6	181.6	186.4	182.8±1.5616		
	Subsurface	3.13	None	152.8	148.8	148.8	148.8	149.8±0.7321	0.9349	3.562
		Dry	None	236.8	238.4	240.0	239.2	238.6±0.4880		
	Subsoil	2.89	None	123.2	121.6	122.4	120.8	122.0±0.3904	0.5187	1.976
		Dry	None	248.8	249.6	251.2	249.6	249.8±0.3416		
Inlet Valley, south of Ithaca, near east valley wall										
Wet	Surface	68.01	4.73	80.0	71.0	73.0	73.0	74.2±1.3908	2.630	10.01
		Dry	4.61	203.8	205.2	195.0	195.7	199.9±2.2322		
	Subsurface	47.83	2.11	25.0	23.0	21.0	21.0	22.5±0.7322	0.8079	3.078
		Dry	2.02	116.8	115.2	114.4	115.2	115.4±0.3416		
Air-dried, laboratory	Surface	2.42	9.45	111.2	111.2	110.4	112.0	111.2±0.1952	0.5255	2.002
		Dry	8.00	239.2	237.6	240.0	240.8	239.4±0.4880		
	Subsurface	2.20	3.34	57.6	55.2	55.2	56.8	56.2±0.4880	0.6901	2.629
		Dry	3.16	143.2	144.0	140.8	143.2	142.8±0.4880		

It will be noted that oven-drying materially increased the total soluble solids in all of the wet samples, air-drying brought about a marked increase and oven-drying the air-dry soil still further increased the total soluble solids, as shown in table 4.

In the first two columns of percentages, the increase is based on the total soluble solids in wet soil but in the third, the percentage increase is based on the total soluble solids in the air-dry soil. This increase calculated on the same basis as the first two is, for the respective samples, 83, 177, 290, 223 and 534 per cent. The increase due to air-drying before oven-drying cannot be due to any nitrification during the slow drying at ordinary temperature, as the increase in nitrates was exceedingly slight, wholly insufficient, calculated as $Ca(NO_3)_2$ to account for the change.

The marked effect of air-drying on these swamp soils may help to account for the relatively high productivity of such soils after a few seasons of cultivation, as against their low productivity when newly turned by the plow.

TABLE 4

Increase in total soluble salts due to drying

SOIL	STRATUM	INCREASE DUE TO OVEN-DRYING WET SOIL	INCREASE DUE TO AIR-DRYING WET SOIL	INCREASE DUE TO OVEN-DRYING AIR-DRY SOIL
		per cent	*per cent*	*per cent*
Drab silt loam near lake	Surface, 0 to 8 inches	60	23	50
	Subsurface, 12 to 20 inches	62	74	59
	Subsoil, 20 to 40 inches	200	94	104
Drab silt loam south of Ithaca	Surface, 0 to 8 inches	167	50	116
	Subsoil, 12 to 20 inches	412	150	154

Kelley and McGeorge (40) state:

> The solubility of soils used in aquatic agriculture is abnormally high, but upon drying out these become much less soluble and approach a state similar to that existing in aerated soils. When such soils are heated after drying, they seem to undergo changes of the same order as are produced in dry-land (ordinary cultivated) soils.

The work here reported, either experiment 3 or 4, is not in accord with their conclusions, as these soils when air-dried, had, in every case, more soluble salts than the same soils when wet.

Experiment 4

The object of this experiment was to study the effects of oven-drying on a wide range of fine-grained soils containing varied amounts of organic matter at three moisture-contents, viz., air-dry, optimum, and water-logged, with the soil kept in the latter two conditions for a period of 9 weeks.

Six soils were used, ranging in texture from clay to sandy loam:

1. *Drab*,[3] or (*Sharkey*,[4]) *clay*. This is a drab colored clay soil, not well supplied with organic matter, stiff and more or less impervious to water, very difficult to work as it is exceedingly tenacious when wet and cloddy when dry. It occurs in large areas in the poorly drained portions of the Mississippi bottom lands, especially south of St. Louis. It is more fully described by Hopkins, Mosier and associates (34), Marbut and associates (65), and as Yazoo clay by Coffey and others (11).

2. *Black clay loam*[5] (Marshall black clay loam). This is a black clay loam soil, well supplied with organic matter. It occurs in flat, depressed areas, former ponds or lakes, and is very productive when thoroughly drained. It is described more fully by Hopkins, Mosier, et al. (33), Marbut (65) and as Miami black clay loam by Coffey, Mosier et al. (12).

3. *Brown silt loam* (Marshall silt loam). This is a brown silt loam soil of good open texture, well supplied with organic matter, and very productive, occupying the rolling prairies of Illinois, Indiana and Iowa. Described by Hopkins, Mosier et al. (35), Marbut (65) and Coffey et al. (13).

TABLE 5

Organic matter contained in these soils*

SOIL	ORGANIC CARBON	TOTAL ORGANIC MATTER	MOISTURE EQUIVALENT
	per cent	*per cent*	*per cent*
1. Drab clay	2.07	3.57	41.6
2. Black clay loam	3.19	5.5	32.4
3. Brown silt loam	2.78	4.8	28.0
4. Yellow-gray silt loam	1.67	2.9	20.7
5. White silt loam	0.435	0.75	
6. Dunkirk fine sandy loam	1.856†	3.20	

* Figures on soils 1 to 4 furnished by Professor J. G. Mosier.

† This determination was kindly made by Dr. F. A. Carlson.

4. *Yellow-gray silt loam* (Knox silt loam). This is a light colored, rolling timber soil, not well supplied with organic matter. More fully described by Hopkins, Mosier et al. (33), Marbut (65) and as Miami silt loam by Coffey, Mosier and others (13).

5. *White silt loam*. This soil was not recognized as a separate type by the Bureau of Soils, as its work in Illinois was done during the beginning of American soil surveying. It is a very light gray silt loam, underlaid by a decidedly impervious stratum, known as "tight clay." It is an unproductive virgin timber soil, very low in organic matter, in fact almost nitrogen free, having but 0.7 per cent of organic matter. This soil is more fully described by Hopkins, Mosier et al. (32).

6. *Sandy loam*. This is the same soil that was used in experiments 1 and 2.

Soils 1 to 5, inclusive, are surface soils, 7 to 8 inches deep. Soils 1 to 4 have been stored in the air-dry state for two or more years in an attic where the humidity is very low and the temperature at times in summer, rather high. Table 5 gives the content of organic matter in these soils.

[3] Name used by Illinois Agricultural Experiment Station.

[4] Name used by United States Bureau of Soils, in Bul. 96, p. 738.

[5] Soils 1 to 5, inclusive, were collected by the writer while a member of the staff of the Agronomy Department, College of Agriculture, University of Illinois.

Organic carbon was determined by the Parr bomb-calorimeter method, CO_2 was measured and reduced to standard P and T from which C was calculated. $C \times 1.724$ (Wolff factor) gives organic matter. This is not claimed to be absolutely accurate nor strictly comparable since the carbon content of soil organic matter varies with its age and the conditions under which decomposition has occurred. The organic matter in white silt loam, for example, is probably more highly carbonized than that in brown silt loam and that in drab clay, a swamp soil, has been affected by a different type of decomposition from brown silt loam, a well-drained type. However, the figure for organic matter is of value in a comparative way when, as in these soils, the differences between them are marked.

Procedure

The soils were air-dried, worked over with a rolling pin, passed through a 2-mm. screen, thoroughly mixed and divided into three portions, *a*, *b*, and *c*. On samples of *a* the usual determinations of total soluble solids and nitrates were made in the original air-dry and in the water-free condition. To *b* distilled water was added, and worked by hand, until it reached what might be termed "optimum" moisture content when the soil was placed in 2-liter earthenware jars. These were weighed on a solution scale the next day and at the end of each succeeding 7-day period, when distilled water was added to restore that lost by evaporation. To *c*, in similar jars, distilled water was added to a point of saturation and more water was poured on as needed to keep the soil submerged. Then *b* and *c* were kept in the laboratory for nine weeks, except the Dunkirk sandy loam which was kept only 8 weeks. The regular determinations were made on both moist and water-free samples at the end of this time.

Results are given in table 6.

Table 6 has several outstanding features. The increase in total solids extracted from soils 1, 2 and 3, well supplied with organic matter, when the original air-dry soil is dried in the oven at 105°C. is very marked; in soils 4, 5 and 6, low in organic matter, the actual increase due to drying is less, but the amount of soluble solids varies from nearly twice to almost three times as much as that in the air-dry soil. In all, except white silt loam, which is very low in organic matter, the moist *b* samples kept 9 weeks at optimum moisture-content had a higher quantity of soluble material than did the air-dry soil, and the dry *b* soil exceeded that water-freed directly from the original air-dry condition. This increase may be accounted for by the increase in nitrates, calculated as $Ca(NO_3)_2$.

The saturated *c* samples in the wet condition showed more total soluble salts than either of the others, even though the nitrates had disappeared. When oven-dried, this soil showed the highest soluble salt content in five of the six soils, and the sixth was but 0.8 mgm. below the next higher soil, the water-freed, moist Dunkirk sandy loam. This may be accounted for in part by the very large amount of water in these soils shown in table 6 to be 75.7 per cent in drab clay down to 37.8 per cent in Dunkirk sandy loam. Here the soil solution is much more dilute and more solids tend to dissolve, as the

solution is probably far from saturated with respect to any of the salts. This soil solution upon adding distilled water to make up the total 500 cc. becomes a part of the 500 cc. and the final result is a more concentrated solution as shown by table 6. When the saturated soil was placed in the oven, water stood on its surface, so the oven-drying process required more than the usual 8 hours. This, it has been suggested, brings more organic matter and much iron and alumina into solution.

Experiment 5. Determination of hygroscopic coefficient

In the hope of securing information which might shed a bit of light on the cause of the increase in soluble material in soils due to heating, two small pieces of experimental work were undertaken, viz. (experiment 5), the determination of the hygroscopic capacity, or coefficient, of the soils used in experiment 4 and (experiment 6) a study of the retention of potassium nitrate by different grades of sand.

The soils are those used in experiment 4 which range from clay to sandy loam.

Procedure

In order to have the soils as uniform as possible, they were passed through a 1-mm. sieve. Duplicate samples of air-dry soil equivalent to approximately 2 gm. of water-free soil were weighed directly into weighing bottles, 7 cm. in diameter. The soil was spread out in a uniformly thin layer over the bottom and exposed to a saturated atmosphere in a humidifier.[6] Strips of filter paper were used as wicks, increasing the surface of contact between air and water in order to insure complete saturation of the air. By means of a water-pump the air pressure within the humidifiers was reduced by several centimeters in order to hasten saturation and reduce condensation. To avoid marked sudden changes in temperature, the humidifiers were placed in a thick-walled wooden box lined with asbestos. This was first located in a cold room and heated electrically, a thermostat being used for maintaining a constant temperature. It was found after 6 weeks' work, however, that the external temperature of winter varied so much that the thermostat and heating apparatus were incapable of maintaining a sufficiently constant temperature. In several instances there was a sudden drop in outdoor temperature the day before weighings were to be made. This brought about condensation in the soil, giving hygroscopic coefficients too high and far from uniform.

The next step was to place the entire apparatus in a deep, unheated basement room. Here the temperature to which the soils were subjected remained fairly constant. The maximum variation in room temperature during the first week was from 61 to 64°F., and inside of the box from 15.5 to 17°C.; the second week the corresponding temperatures were 60.5 to 65.5°F. for the room and from 15 to 18°C. inside the box. There did not appear to be any condensation and yet there is not an altogether satisfactory agreement of duplicate determinations made the first week on soils 4, 5 and 6.

The soils were kept in the saturated atmosphere for a period of 7 days when the humidifiers were removed from the constant-temperature box. Before removing the lid of humidifiers, the pressure was equalized by admitting air *very slowly* through the side tube. Upon removing the weighing bottles from the humidifier, the lids were immediately and tightly inserted

[6] The humidifier was a large desiccator whose dehydrating substance had been replaced by a 10 per cent solution of sulfuric acid to furnish the water vapor. The desiccators are supplied with side tube and stop cock.

TABLE 6

Effect of moisture content on soluble material in soils

CONDITION	MOISTURE CONTENT	NITRATES	TOTAL SALTS RECOVERED				MEAN	E	E × 3.81
			I		II				
			A	B	A	B			
Drab clay									
	per cent	*mgm.*	*mgm*	*mgm.*	*mgm.*	*mgm.*			
Air dry	5.59	4.00	68.0	68.0	67.2	68.8	68.0±0.1952	0.8998	3.428
	Dry	5.00	150.4	145.6	150.4	148.0	148.6±0.8784		
Optimum moisture	25.98	36.20	82.4	82.4	81.6	80.8	81.8±0.2928	0.3171	1.208
	Dry	30.60	162.4	163.2	163.0	163.2	163.0±0.1220		
Saturated	75.75	None	131.2	130.4	128.8	128.0	129.6±0.5856	0.6343	2.417
	Dry	None	168.0	167.2	166.4	166.8	167.1±0.2440		
Black clay loam									
Air-dry	5.15	2.52	72.8	72.8	71.2	70.4	71.8±0.4880	0.9001	3.429
	Dry	3.88	165.6	167.2	169.6	169.6	168.0±0.7564		
Optimum moisture	20.82	22.30	76.8	76.0	75.2	76.8	76.2±0.2928	0.3812	1.452
	Dry	17.81	183.2	184.8	184.0	184.4	184.1±0.2440		
Saturated	69.88	None	95.2	96.0	96.0	96.8	96.0±0.1952	0.8998	3.428
	Dry	None	200.0	198.4	195.2	196.0	197.4±0.8784		
Brown silt loam									
Air-dry	2.33	6.25	82.4	83.2	83.2	81.6	82.6±0.2928	0.9259	3.528
	Dry	5.54	180.8	181.6	176.0	179.2	179.4±0.8784		
Optimum moisture	21.80	44.18	98.0	98.4	97.6	96.0	97.5±0.3661	0.9178	3.497
	Dry	43.54	200.0	204.4	199.2	200.0	200.9±0.8541		
Saturated	48.74	None	115.2	118.0	116.8	115.2	116.3±0.5368	1.1996	4.570
	Dry	None	203.2	204.0	198.4	200.0	201.4±1.0736		

Yellow gray silt loam									
Air-dry	1.24	4.86	53.6	53.6	52.0	52.8	53.0±0.2928	0.4141	1.578
	Dry	4.44	96.8	95.2	95.2	96.0	95.8±0.2928		
Optimum moisture	16.85	32.32	71.2	70.4	69.6	72.0	70.8±0.3904	0.6637	2.539
	Dry	28.50	116.8	114.8	114.4	116.8	115.7±0.5368		
Saturated	44.67	None	73.6	72.8	72.8	71.2	72.6±0.3416	0.5956	2.269
	Dry	None	123.2	122.4	124.0	120.8	122.6±0.4880		
White silt loam									
Air-dry	1.25	Faint trace	34.4	34.8	33.6	35.2	34.5±0.2440	0.4603	1.754
	Dry	None	93.6	92.0	95.2	93.6	93.6±0.3904		
Optimum moisture	18.16	0.8	26.4	26.4	26.4	24.8	26.0±0.2928	0.4499	1.714
	Dry	0.2	94.4	93.6	95.2	95.6	94.7±0.3416		
Saturated	39.88	None	73.6	73.6	75.2	72.8	73.8±0.3416	0.4831	1.840
	Dry	None	100.0	101.6	100.0	99.2	100.2±0.3416		
Dunkirk sandy loam									
Air-dry	0.90	22.70	68.0	66.4	66.4	66.4	66.8±0.2928	0.8339	3.177
	Dry	18.76	120.8	122.4	124.8	124.8	123.2±0.7808		
Optimum moisture	12.41	37.58	77.6	76.8	76.8	78.4	77.4±0.2928	1.0658	4.061
	Dry	28.50	132.8	132.0	126.4	131.2	130.8±1.0248		
Saturated	37.80	None	48.8	50.4	48.8	49.6	49.4±0.2928	0.4880	2.629
	Dry	None	129.6	128.8	130.4	131.2	130.0±0.3904		

to prevent evaporation and the bottles with soil weighed at once after wiping them perfectly dry. After weighing, the soil was dried in the bottles at 105°C. for 8 hours and again weighed. The loss is the hygroscopic moisture or, expressed as per cent on the basis of oven-dry soil, the hygroscopic coefficient.

The results of the determinations appear in table 7.

As pointed out by Beaumont (3, page 492–493), the probable error of determinations of hygroscopic coefficient is high. The accuracy of this statement is well borne out by the figures in table 7. His results indicated that slight variations in temperature made little difference in adsorption of water vapor so the only effort in this work was to keep the temperature sufficiently constant to prevent condensation of free water on the particles, or in the inter-

TABLE 7

Hygroscopic coefficients of air-dry soils used in experiment 4

SOIL	WATER ABSORBED IN 7 DAYS				MEAN	HYGROSCOPIC MOISTURE	ORGANIC MATTER	TOTAL SOLUBLE SALTS AT OPTIMUM MOISTURE*	AVERAGE SOLUBLE-SALT CONTENT OF AIR-DRY, OPTIMUM-MOISTURE, AND WATER-FREE SOILS*
	First trial		Second trial						
	A	B	A	B					
						per cent of dry soil	*per cent*	*mgm.*	*mgm.*
1. Drab clay	11.66	11.62	11.30	11.62	11.550±0.061	5.59	3.57	81.8	115.4
2. Black clay loam	9.95	10.24	9.94	10.02	10.037±0.493	5.15	5.50	76.2	125.0
3. Brown silt loam	4.45	4.62	4.41	4.47	4.487±0.0322	2.33	4.80	97.5	140.1
4. Yellow gray silt loam	2.05	2.50	2.30	2.26	2.277±0.0598	1.24	2.90	70.8	83.8
5. White silt loam	1.55	1.95	1.85	1.83	1.795±0.0299	1.25	0.75	26.0	62.2
6. Dunkirk fine sandy loam	1.95	2.44	2.22	2.48	2.272±0.0915	0.90	3.20	77.4	99.5

* Data taken from table 6.

stices of the soil. This latter seems to have occurred during the early part of this work as the hygroscopic coefficient was high and quite erratic. These figures seem to bear out Beaumont's conclusion, also, that other factors than total surface affect the adsorption of water. Soils 4 and 5 are very similar in texture, consequently in total surface, and the hygroscopic moisture actually in the soils, air-dry, is the same, yet when exposed to the same saturated atmosphere, soil 4, with higher organic-matter and soluble-salt content, adsorbs 0.482 per cent more actual moisture, 27 per cent excess over soil 5, the one of lower salt- and organic-content. In this connection, let us compare soils 5 and 6, in table 7. White silt loam has much more surface than Dunkirk fine sandy loam.[7] In the air-dry state they appear in their right order as

[7] No mechanical analysis available.

regards hygroscopic moisture and surface having, respectively, 1.25 and 0.90 per cent hygroscopic moisture. However, when exposed to a saturated atmosphere for 7 days, they stand in a different relationship to each other, their positions being reversed, and instead of the soil with greater surface absorbing more moisture, more is taken up by the soil which has less surface, but contains more soluble salts. This tends to show that within certain limits, at least, soluble salts are of sufficient importance to reverse the normal effect of total surface exposed by a soil.

Considering table 7 in a broad, general way, the hygroscopic moisture content in the air-dry soil bears a normal relationship to the total surface, and when we consider surface and organic-matter content this same general relationship holds for hygroscopic coefficient except in case of the sandy loam where the discrepancy is due to its large quantity of soluble salts. The difficulty here lies in that we have not a single variable factor, but several, viz., size of particles, total surface, organic matter, and soluble salts.

Since it is almost, if not wholly impossible to control all factors at once, it may be necessary to work with synthetic soils, varying but a single factor at a time. These determinations of hygroscopic coefficients cannot yield much valuable evidence, except that, in a general way, the soils with the highest percentage of clay and colloids have the highest hygroscopic coefficients and within the limits of this experiment have the highest soluble salt content. However, the data are so meagre that no definite safe conclusion may be drawn.

Experiment 6. Retention of nitrate by quartz and white silt loam

The purpose of this experiment was to study the question whether clean quartz sand holds potassium nitrate, and if so, to what extent. This salt was selected because the acid radical is not generally supposed to be adsorbed to an appreciable degree, and also because it was available as a chemically pure substance.

The material used was a clean ground quartz. It was first leached with a 10 per cent solution of hydrochloric acid to remove any possible soluble material, then washed free of the acid and air-dried. It was then sifted into four grades, as used by the United States Bureau of Soils:

	Diameter mm.
Coarse sand	1.00–0.50
Medium sand	0.50–0.25
Fine sand	0.25–0.10
Very fine sand	0.10–0.05

White silt loam from experiments 4 and 5 was used also, since in the air-dry condition it was essentially devoid of nitrates.

The water-holding capacity of 50 gm. of each of these materials was then determined experimentally. Water was added from a burette and the materials were found to hold, without any loss by percolation on standing, the

following quantities: coarse sand, 10 cc.; medium sand, 10 cc.; fine sand, 15 cc.; very fine sand, 17 cc.; white silt loam, 18 cc.

According to Mosier and Gustafson (68) the surface per gram of the different grades, assuming perfect spheres of average diameter for the grades, is as follows: coarse sand, 30.2 sq. cm.; medium sand, 60.4 sq. cm.; fine sand, 129.3 sq. cm.; very fine sand, 302.1 sq. cm.; white silt loam, 2270.7 sq. cm. Analysis[8] of this latter soil shows (Bureau of Soils sizes) 1.5 per cent medium sand and coarser grades, 1.7 per cent fine sand, 7.5 per cent very fine sand, 70.6 per cent silt, and 18.3 per cent clay. The calculation of the surface per gram of white silt loam was based upon these percentages, the surface areas given above, and the assumption that 824.8 sq. cm. is the surface of 1 gm. of silt and that 9090.2 sq. cm. is the surface of 1 gm. of clay.

The figures for total surface per gram are undoubtedly more nearly accurate for white silt loam than for the quartz since the latter is angular and of all conceivable shapes with no spheres. The surface of the quartz must be considerably greater than the figures show, whereas in the white silt loam soil the angles have been worn off the particles to some extent, bringing them somewhat toward the spherical shape—yet the figures at best are but an approximation which may be of some value for purposes of discussion.

Solutions of potassium nitrate were then made up of such strength that 1 cc. of the first solution contained 0.1 mgm. of nitrate and the other, 0.5 mgm. of nitrate. The hygroscopic capacity of quartz is so low that this factor was ignored. A quantity of potassium nitrate solution equal to the water-holding capacity was added from an accurate burette to each of four 50-gm. samples of the grades of quartz and of white silt loam. These moistened samples were covered to prevent evaporation and set aside over night to permit of any reaction or adjustment in the moist mass, after which two samples of each were placed in the oven at 105°C. for 8 hours. Nitrates were determined immediately on the other two samples. Each was placed in a 500-cc. beaker and distilled water added to make a total of 250 cc.; for example, to the coarse sand containing 10 cc. of KNO_3 solution, 240 cc. of distilled water was added and to the very fine sand having 17 cc. of the solution, 233 cc. of water was added. Thus the relationship throughout was 1 part of soil or quartz to 5 of water.

This experiment was run in two parts, 6a and 6b. In the first part, the nitrate solution used contained 100 parts per million, or 0.1 mgm. per cubic centimeter of nitrate and in the second, the solution contained 500 parts per million or 0.5 mgm. per cubic centimeter. In all other respects the two trials were identical. The stronger solution was used in the second trial because a dilution of 10 cc. of the first solution to 250 cc. was considered too great for securing results of the degree of accuracy desired.

The results of the first set of determinations are given in table 8.

The results of the second set of determinations are given in table 9.

[8] This analysis was kindly furnished by Dr. Bizzell and Dr. Buckman.

TABLE 8

Nitrate recovered from ground quartz and white silt loam

(Nitrate solution, 100 p. p. m. or 0.1 mgm. per cc.)

SIZE OF MATERIAL	CONDITION	AMOUNT NITRATE ADDED	FIRST EXTRACTION		SECOND EXTRACTION		THIRD EXTRACTION		RECOVERED BY FIRST EXTRACTION
			NO_3 found	NO_3 in 250 cc.	NO_3 found	NO_3 in 250 cc.	NO_3 found	NO_3 in 250 cc.	
		mgm.	*p. p. m.**	*mgm.**	*p. p. m.**	*mgm.*	*p. p. m.**	*mgm.*	*per cent*
Coarse sand	Moist	1.0	3.22	0.805	0.48	0.12	0.33	0.08	80.50
	Dry	1.0	3.15	0.79	Trace	Trace	0.36	0.09	79.00
Medium sand	Moist	1.0	3.17	0.79	0.56	0.14	0.32	0.08	79.00
	Dry	1.0	2.82	0.705	Trace	Trace	0.32	0.08	70.50
Fine sand	Moist	1.5	4.67	1.17	1.03	0.26	0.42	0.10	78.00
	Dry	1.5	4.11	1.03	0.62	0.15	Trace	Trace	68.67
Very fine sand	Moist	1.7	4.59	1.15	0.96	0.24	Trace	Trace	67.64
	Dry	1.7	4.41	1.10	0.71	0.18	0.25	0.06	64.70
White silt loam	Moist	1.8	5.57†	1.393					77.40

* Average of four determinations.

† Average of eight determinations.

TABLE 9

Nitrate recovered from ground quartz

(Nitrate solution, 500 p. p. m. or 0.5 mgm. per cc.)

SIZE OF MATERIAL	CONDITION	AMOUNT OF NITRATE ADDED	FIRST EXTRACTION			SECOND EXTRACTION			TOTAL NO_3 RECOVERED
			NO_3 found	NO_3 in 250 cc. if homogeneous		NO_3 found	NO_3 in 250 cc. if homogeneous		
		mgm.	*p. p. m.**	*mgm.*	*per cent*	*p. p. m.**	*mgm.*	*per cent*	*per cent*
Coarse sand	Moist	5.0	17.74	4.435	88.7	1.21	0.302	6.0	94.7
	Dry		15.30	3.825	76.5	0.49	0.122	2.4	78.9
Medium sand	Moist	5.0	15.45	3.886	77.5	1.103	0.276	5.5	83.0
	Dry		14.75	3.687	73.7	0.48	0.120	2.4	76.1
Fine sand	Moist	7.5	28.51	7.127	95.0	1.56	0.390	5.2	100.2
	Dry		27.27	6.817	91.0	0.48	0.120	1.6	92.6
Very fine sand	Moist	8.5	30.78	7.695	90.6	2.06	0.515	6.0	96.6
	Dry		29.26	7.315	86.0	0.703	0.176	2.3	88.3

* Average of four determinations.

A glance at tables 8 and 9 shows that the recovery of nitrate was less complete with the more dilute solution, the average percentage of recovery being 76.3 for the moist quartz and 70.7 for the oven-dry quartz, compared

TABLE 10

Nitrate removed from four grades of ground quartz

SIZE OF MATERIAL	CONDITION	NITRATE			SOLUTION REMAINING IN QUARTZ	NITRATE		SOLUTION REMAINING IN QUARTZ	NITRATE REMOVED		EXTRACTION OF SAND-NITRATE		NITRATE ACCOUNTED FOR
		Amount added	First extraction			Second extraction			First extraction	Second extraction			
			Found	Actually removed		Found	Actually removed						
		mgm.	*p. p. m.*	*mgm.*	*cc.*	*p. p. m.*	*mgm.*	*cc.*	*per cent*	*per cent*	*mgm.*	*per cent*	*per cent*
Coarse sand	Moist	5.0	17.74	4.183	14.18	1.21	0.286	14.37	83.6	5.7	0.625	12.5	101.8
	Dry	5.0	15.30	3.583	15.84	0.49	0.115	15.84*	71.6	2.3	Trace		78.9
Medium sand	Moist	5.0	15.545	3.675	13.53	1.103	0.259	14.8	73.5	5.2	1.023	20.46	99.2
	Dry	5.0	14.75	3.478	14.23	0.48	0.113	14.23*	69.5	2.3	Trace		76.1
Fine sand	Moist	7.5	28.51	6.680	15.72	1.56	0.368	14.06	89.0	7.5	0.917	10.79	107.2
	Dry	7.5	27.27	6.403	15.23	0.48	0.113	16.23*	85.3	2.3	Trace		92.6
Very fine sand	Moist	8.5	30.78	7.187	16.54	2.06	0.480	17.15	84.6	9.6	0.826	9.7	103.9
	Dry	8.5	29.26	6.809	17.25	0.703	0.164	17.25*	80.1	3.5	Trace		88.3

* Weight not taken. Assumed to be same as after first extraction.

with 87.9 and 81.8 which are the corresponding figures for the more concentrated solution. Thus it is seen that the recovery from the oven-dry quartz was less in both cases, less by 5.6 per cent for the dilute solution and by 6.1 per cent for the more concentrated solution. This relationship holds throughout the second extraction also. This seems to indicate a real loss of nitrate due to heating in the oven for 8 hours at 105°C.

In order to test this point further, eight 10-cc. samples of a nitrate solution containing 100 parts per million and eight 5-cc. portions containing 500 parts per million were treated with a few drops of saturated sodium carbonate solution and evaporated to dryness on the water-bath. The nitrates in four samples of each were determined at once in the usual manner. The remaining four samples of each concentration were dried in the oven at about 105°C. for 8 and 21 hours, respectively. The results of all the determinations are given in table 11.

TABLE 11

Effect of heating at 105°C. on quantity of nitrate recovered from quartz sand

GRADE OF MATERIAL	CONCENTRATION			
	About 0.1 mgm. per cubic centimeter		About 0.5 mgm. per cubic centimeter	
	Not heated	After heating	Not heated	After heating
Coarse sand	40.125	43.75*	36.00	41.00
Medium sand	40.375	45.20*	37.25	41.00
Fine sand	40.750	45.40*	37.00	40.25
Very fine sand	40.000		37.25	40.50
Average	40.31	44.59	36.87	40.69
Percentage recovery	100.00	90.39	100.00	90.86

* Average of five readings, others average of four readings.

Since all of the above were compared with the same standard and all samples of each concentration were diluted to the same extent, the average colorimeter readings bear to each other exactly the same relationship as would the actual milligrams of nitrate, so the reading of unheated samples is considered 100 and the other found thus: 44.59 : 40.31 = 100 : x. Of the weaker solution, 10 cc. was diluted to 1000 cc. and of the stronger, 5 cc., to 2500 cc. in order to bring each to approximately 1 part per million for reading. From these figures the total milligrams of nitrate in each can be calculated. These figures show a loss of nearly 10 per cent of the nitrate after the period of heating at slightly above the boiling point. This is in accord with the earlier work recorded in this paper. For this reason further discussion here will deal with the unheated materials only.

In the case of the coarse and medium grades with the same quantity of nitrate added, the medium holds more, or gives up less, nitrate at both

concentrations; the same relationship holds at both concentrations when we compare the nitrate recovered from the fine and very fine grades receiving, respectively, 15 and 17 cc. of nitrate solution. This tends to show that the difference is due to a surface effect.

Let us consider the very fine sand grade in tables 9 and 10. There was incorporated in this grade 8.5 mgm. of nitrate. The first extraction removed 7.187 mgm. of nitrate in the 233.46 cc. of drainage (table 10). Had the entire 250 cc. of diluted nitrate solution been a homogeneous mixture, this quantity of solution could have contained only 7.695 mgm. of the original 8.5 mgm. incorporated.

In the second extraction of this grade, 0.48 mgm. of the 1.313 mgm. actually remaining in the quartz, was removed in the 232.85 cc. of drainage. Had this second solution been homogeneous, 0.515 mgm. of nitrate would have been recovered, which still leaves 0.798[9] mgm. of nitrate to be accounted for.

How shall we account for the apparent failure of 0.805 mgm. of nitrate to go into solution in the first, and 0.798 mgm. in second extraction? To the writer there seems but one answer to this question, that of King (46). When the nitrate solution came in contact with the quartz particles, a definite attraction due to the force of adhesion was set up. The quantity of the more concentrated solution added was greater than the sand could hold by adsorption, so that when the distilled water was supplied the nitrate solution held mechanically only, formed a homogeneous mixture with the water when stirred. The 16.54 cc. of solution remaining in the quartz after the first extraction, if of the same composition as the 233.46 cc. removed, would have contained 0.508 mgm. of nitrate. This is 0.028 mgm. in excess of the 0.48 mgm. actually removed in the second extraction, or, 0.007 mgm. less than would have been contained in the 250 cc. of solution had it been of the same composition as that draining from the sand.

Since the dilution is so much greater in the second extraction, it may be expected that the outer part of the film, less strongly adhering to the particles would tend to form a homogeneous mixture with the water, this together with the nitrate solution held mechanically only, and that coming by diffusion from the strongly held film, furnishes the whole of the nitrate removed in the second extraction. King's data, table A (p. 177), shows that after six extractions the nitrate recovered is that coming from the strongly adhering film by diffusion.

Five months after the two extractions were made, the air-dry quartz samples which had stood in the laboratory in beakers covered with paper were themselves treated with phenoldisulfonic acid and nitrates determined in the usual way. The nitrate so determined is shown as milligrams and per cent of the total in the latter part of table 10. In no case did the samples which had been heated in the previous extractions show the presence of nitrates, while all

[9] 8.5 mgm. total; actually removed by first extraction, 7.187 mgm., or 1.313 mgm. remained; $1.313 - 0.515 = 0.798$ mgm.

unheated samples contained considerable quantities of nitrates. No claim is made that the conditions under which these had been stored are such that the nitrate now recovered, together with that previously removed, should equal 100 per cent of the original quantity of nitrate used. However, it is notable that in all except the fine sand size the total recovery is nearly 100 per cent.

These data are not in accord with those of Noyes (71) who reports all nitrate recovered in one extraction nor with those of Bouyoucos (6), published since the above was written, who states that the soil solution is less concentrated at the immediate surface of the particles.

Under the conditions of this experiment, not all of the nitrate added to pure quartz nor to a soil containing but a faint trace of nitrate (white silt loam, table 6), was recovered in one extraction nor even in two extractions. While the data here reported are entirely too meager to warrant definite conclusions, yet it is significant that extracting the quartz itself brings the nitrate recovered so near 100 per cent of the total added.

This work corroborates in every important detail that of King (46) and though these data are meager they indicate clearly that with soils containing a moderately low percentage of capillary moisture, at, or slightly below optimum, much of the soluble salts are held in this film moisture. This film adheres so strongly to the particle that much of the soluble material it contains is given up to water on extraction by diffusion only and this explains, in part, the effect of air-drying. When the capillary moisture is lost the salts are left as minute crystals on the soil particles, and it is clear that quickly washing with water will bring these salts into solution so as to give a more homogeneous solution than can be obtained by washing a moderately moist soil in the same way.

When a soil is oven-dried we get all of the effect of air-drying just discussed, together with several additional effects. Heating at 105°C. coagulates the colloids. This the oven-dry samples showed very clearly, on working them with a pestle in the mortar. With the heavier soils much difficulty was encountered in making a "thin paste," as the soil adhered so tightly to both the pestle and the mortar. After heating, there was no tenacity whatever, a heavy silt or clay loam working as easily as did fine sandy loam. This action on the colloidal matter would enable the water to come into more intimate contact with the material which exposes the major part of the total surface of the soil. As already shown, Hulett and Allen (37), the particles of colloidal size are more soluble than larger ones. This effect on colloids is undoubtedly an important factor, and helps explain higher solubility in the soils with smallest particles.

Authors cited above hold that heating alters organic as well as inorganic materials in the soil, rendering them more soluble. While the physical factor in sands and silt loam has been shown to explain in part the increased recovery of salts due to heating, it seems to the writer that the effect on colloids and the well-known effect of heat in increasing the solubility of some minerals and organic matter also are important factors.

SUMMARY

1. Oven-drying increased to a marked degree the quantity of water-soluble material removed from soil by 1:5 extraction with distilled water.

2. Air-drying swamp soils increased the water-soluble material and oven-drying this air-dried soil brought about a still further increase.

3. Storing soils for 9 weeks at 8 to 12°C. in open jars (in which water evaporated was restored each week) or in sealed tubes in its original condition did not markedly affect the total soluble material. Nitrification occurred in the open jars, while nitrates decreased, as a rule, in the sealed tubes.

4. Keeping soil at room temperature and optimum moisture content for 9 weeks did not materially affect the amount of soluble material, but there was a slight increase in all soils except white silt loam. Keeping these soils saturated at the same temperature, greatly increased the soluble material. In the first case nitrification was active while in the latter, denitrification was complete.

5. Oven-drying decreased the nitrate-content of these soils.

6. When potassium nitrate in two concentrations was added to four grades of quartz, it was not wholly recovered in one, or even two 1:5 extractions. From the more dilute solution 67 to 80 per cent of the nitrate were recovered in one extraction, while from the more concentrated, 77 to 95 per cent were recovered.

7. When potassium nitrate in a dish is heated in an oven at 105°C. for 8 hours, after being evaporated to dryness, a distinct loss of nitrate occurs.

GENERAL CONCLUSIONS

The literature cited and the experimental work here reported both bring out one important point. They show the effect of drying at room temperature, and heating in an oven at slightly above the boiling point, and in the autoclave at various pressures and temperatures. In general, the quantity of soluble inorganic constituents and organic matter is increased, while temperatures above 100°C. reduce the quantity of nitrates. These facts indicate that in planning soil biology studies, pot-culture or other greenhouse fertility investigations, the soils used should be kept under conditions strictly comparable as to aeration, moisture content and temperature in order to avoid the introduction of uncontrolled factors which might lead to erroneous conclusions.

REFERENCES

(1) AITKEN, J. 1910 The fertilizing influence of sunlight. *In* Nature (London), v. 83, p. 37.

(2) ALLEN, E. R., AND BONAZZI, A. 1915 On nitrification. Ohio Agr. Exp. Sta. Tech. Bul. 7, p. 37.

(3) BEAUMONT, A. B. 1919 Studies in the reversibility of the colloidal condition of soils. N. Y. (Cornell) Agr. Exp. Sta. Mem. 21, p. 479–524.

(4) BONSTEEL, J. A., FIPPIN, E. O. AND CARTER, W. T. 1905 Soil survey of Tompkins County, N. Y. U. S. Dept. Agr., Field Operations Bur. Soils, 7th Rpt., p. 29.

(5) BOUYOUCOS, G. J. 1915 The freezing point method as a new means of measuring the concentration of the soil solution directly in the soil. Mich. Agr. Exp. Sta. Tech. Bul. 24, p. 31–32.

(6) BOUYOUCOS, G. J. 1921 The concentration of the soil solution around the soil particles. *In* Soil Sci., v. 11, p. 131–138.

(7) BUCK, E. 1915 Ráb: A unique system of cultivating rice in western India. *In* Internat. Inst. Agr. (Rome) Mo. Bul. Agr. Intel. and Plant Diseases, v. 6, p. 1111–1117. *Abs. in* Exp. Sta. Rec. (1916), v. 35, p. 138.

(8) BUDDIN, WALTER 1914 Note on the increased nitrate content of soil subjected to temporary drying in the laboratory. *In* Jour. Agr. Sci., v. 6, p. 452–455.

(9) CARD, F. W., AND BLAKE, M. A. 1905 Report of the Horticultural Division. *In* R. I. Agr. Exp. Sta. Ann. Rpt. 1905, p. 197–219.

(10) CHRISTENSEN, H. R. 1917 Experiments in methods for determining reaction of soils. *In* Soil Sci., v. 4, p. 115–178.

(11) COFFEY, G. N. AND PARTY 1902 Soil Survey of St. Clair County, Ill. U. S. Dept. Agr., Field Operations Bur. Soils, 4th Rpt., p. 85.

(12) COFFEY, G. N., ELY, C. W., MANN, C. J., et al. 1903 Soil Survey of McLean County, Ill. U. S. Dept. Agr., Field Operations Bur. Soils, 5th Rpt., p. 785.

(13) COFFEY, G. N., ELY, C. W., et al. 1903 Soil Survey of Sangamon County, Ill. U. S. Dept. Agr., Field Operations Bur. Soils, 5th Rpt., p. 710–712.

(14) COLEMAN, D. A., LINT, H. C., AND KOPELOFF, N. 1916 Can soil be sterilized without radical alteration? *In* Soil Sci., v. 1, p. 259–274.

(15) CONNOR, S. D. 1916 Acid soils and the effect of acid phosphate and other fertilizers upon them. *In* Jour. Indus. Engin. Chem., v. 8, p. 35–40.

(16) DARBISHIRE, F. V., AND RUSSELL, E. J. 1907 Oxidation in soils and its relation to productiveness. *In* Jour. Agr. Sci., v. 2, p. 305–326.

(17) DAVY, SIR HUMPHREY 1813 Elements of Agricultural Chemistry. London. p. 299.

(18) DEHÉRAIN, P. P., AND DEMOUSSY, M. E. 1896 Sur l'oxydation de la matiére organic du sol. *In* Ann. Agron., v. 22, p. 305–337.

(19) DIETRICH, TH. 1901 Versuche über den Einfluss der Bodensterilisation auf das Wachstum der in dem sterilisierten Boden kultivierten Pflanzen. *In* Jahresb. Landw. Vers. Marburg, 1901–2, p. 16. *Abs. in* Centbl. Agr. Chem. (1903), p. 68 and *in* Jour. Agr. Sci. v, 2, p. 312.

(20) DYER, B. 1910 Fertilizing effect of soil sterilization. *In* Nature (London), v. 83, p. 96.

(21) EHRENBERG, PAUL 1915 Die Bodenkolloide, p. 40–201. Koehler and Volckmer, Leipzig.

(22) FISCHER, HUGO 1912 Vom Trochnen des Bodens. *In* Centbl. Bakt. (etc.), Abt. 2, v. 36, p. 346–349.

(23) FLETCHER, P. 1910 The fertilizing influence of sunlight. *In* Nature (London), v. 83, p. 156–157.

(24) FRANK, B. 1888 Ueber den Einfluss, welchem des Steriliziren des Erdbodens auf die Pflanzen-entwickelung ausübt. *In* Ber. Deut. Bot. Gesell. (General versamlungs Heft), v. 6, p. 87–97.

(25) GEDROITZ, K. K. 1909 Influence of sterilization of the soil on the growth of plants on the soil. *In* Trudui Selsk. Khoz. Khim. Lab. St. Petersb., v. 6, p. 304–342. *Abs. in* Exp. Sta. Rec., v. 23, p. 221.

(26) HALL, A. D. 1910 The fertility of the soil. *In* Science, n. s., v. 32, p. 363–371.

(27) HALL, THOMAS DENNISON 1915 A study in wetting and drying the soil. Unpublished thesis, Cornell University, p. 1–30.

(28) HARTWELL, B. L., AND PEMBER, F. R. 1918 The presence of aluminum as a reason for the difference in the effect of so-called acid soil on barley and rye. *In* Soil Sci., v. 6, p. 259–280.

(29) HASENBÄUMER, J., COPPENRATH, E., AND KÖNIG, J. 1905 Einige neue Eigenschaften des Ackerbodens. *In* Landw. Vers. Stat., v. 63, p. 471–478.

(30) HILGARD, E. W. 1906 Soils, p. 321–331. MacMillan, New York.

(31) HINSON, W. M., AND JENKINS, E. H. 1910 The management of tobacco seed beds. Conn. Agr. Exp. Sta. Bul. 166.

(32) HOPKINS, C. G., MOSIER, J. G., PETTIT, J. H., AND FISCHER, O. S. 1913 Bond County soils. Ill. Agr. Exp. Sta. Soil Rpt. 8, p. 41–42.

(33) HOPKINS, C. G., MOSIER, J. G., PETTIT, J. H., AND READHIMER, J. E. 1912 Sangamon County soils. Ill. Agr. Exp. Sta. Soil Rpt. 4, p. 21 and 23–34.

(34) HOPKINS, C. G., MOSIER, J. G., VAN ALSTINE, E., AND GARRETT, F. W. 1913 Pike County soils. Ill. Agr. Exp. Sta. Soil. Rpt. 11, p. 33.

(35) HOPKINS, C. G., MOSIER, J. G., VAN ALSTINE, E., AND GARRETT, F. W. 1918 Champaign County soils. Ill. Agr. Exp. Sta. Soil Rpt. 18, p. 27.

(36) HOWARD, A., AND HOWARD, G. L. C. 1909–10 The fertilizing influence of sunlight. *In* Nature (London), v. 82, p. 456–457.

(37) HULETT, G. A., AND ALLEN, L. E. 1902 The solubility of gypsum. *In* Jour. Amer. Chem. Soc. v. 24, p. 667–679.

(38) JOHNSON, JAMES. 1916 Preliminary studies on heated soils. *In* Science, n. s., v. 43, p. 434–435.

(39) JOHNSON, JAMES 1919 The influence of heated soils on seed germination and plant growth. *In* Soil Sci., v. 7, p. 1–104.

(40) KELLEY, W. P., AND MCGEORGE, W. 1913 The effect of heat on Hawaiian soils. Hawaii Agr. Exp. Sta. Bul. 30, p. 5–38.

(41) KELLEY, W. P., AND THOMPSON, A. R. 1915 The organic nitrogen of Hawaiian soils. II: Effects of heat on soil nitrogen. *In* Jour. Amer. Chem. Soc., v. 36, p. 434–438.

(42) KING, F. H. 1901 Development and distribution of nitrates and other soluble salts in cultivated soils. Wis. Agr. Exp. Sta. Bul. 85, p. 1–48.

(43) KING, F. H. 1904 Promising methods for the investigation of problems of soil and plant physiology, and some lines of investigation to which they are adapted. *In* Proc. Soc. Prom. Agr. Sci. (1904), p. 171–190.

(44) KING, F. H. 1904 Investigations in Soil Management. Influence of Soil Management upon the Water-Soluble Salts in Soils and the Yield of Crops, p. 1–168. F. H. King, Madison, Wis.

(45) KING, F. H. 1905 Investigations in soil management. U. S. Dept. Agr. Bur. Soils Bul. 26, p. 1–124.

(46) KING, F. H. 1909 The suspension of solids in fluids and the nature of colloids and solutions. Sep. from Trans. Wis. Acad. Sci., Arts and Letters 16 (1908), pt. 1, p. 275–288. *Abs. in* Exp. Sta. Rec., v. 21, p. 19.

(47) KING, F. H. 1911 Farmers of Forty Centuries, p. 142–143. F. H. King, Madison, Wis.

(48) KING, F. H. 1914 Soil Management, p. 297–299. Orange Judd Co., New York.

(49) KING, F. H., AND JEFFREY, J. A. 1899 The soluble salts of cultivated soils. Wis. Agr. Exp. Sta. 16th Ann. Rpt., p. 219–242.

(50) KING, F. H., AND WHITSON, A. R. 1900 Soluble salts in cultivated soils. Wis. Agr. Exp. Sta., 17th Ann. Rpt., p. 204–226.

(51) KING, F. H., AND WHITSON, A. R. 1901 Development and distribution of nitrates in cultivated field soils. Wis. Agr. Exp. Sta. 18th Ann. Rpt., p. 210–231.

(52) KING, F. H., AND WHITSON, A. R. 1902 Development and distribution of nitrates in cultivated soils. Wis. Agr. Exp. Sta. Bul. 93, p. 1–39.

(53) KLEIN, MILLARD, A. 1915 Studies in drying of soils. *In* Jour. Amer. Soc. Agron., v. 7, p. 49–77.

(54) KOCH, G. P. 1917 The effect of sterilization of soils by heat and antiseptics upon the concentration of the soil solution. *In* Soil Sci., v. 3, p. 525–530.

(55) KOCH, A., AND LÜKEN, G. 1907 Ueber die Veränderung eines leichten Sandbodens durch Sterilisation. *In* Jour. Landw., v. 55, p. 161–172.

(56) KÖNIG, J., HASENBÄUMER, J. AND GLENK, K. 1913 Ueber die Anwendung der Dialyse und die Bestimmung der Oxydationskraft für die Beurteilung des Bodens. *In* Landw. Vers. Stat., v. 79–80, p. 491–534.

(57) KOPELOFF, N., AND COLEMAN, D. A. 1917 A review of investigations in soil protozoa and soil sterilization. *In* Soil Sci., v. 3, no. 3, p. 197–269.

(58) KRÜGER AND SCHNEIDEWIND W. 1899 Ursache und Bedeutung der Salzpeterzersetzung im Boden. *In* Landw. Jahrb., v. 28, p. 216–252.

(59) LEATHER, J. W. 1912 Records of drainage in India. Mem. Dept. Agr. India, v. 2, p. 63–140.

(60) LIEBSCHER, G. 1893 Deut. Landw. Presse, no. 94, p. 976. *Abs. in* Jour. Agr. Sci., v. 2, p. 311.

(61) LYON, T. L., AND BIZZELL, J. A. 1909 Changes produced in soils by subjecting them to steam under pressure. *Abs. in* Jour. Soc. Chem. Indus., v. 28, p. 721.

(62) LYON, T. L., AND BIZZELL, J. A. 1910 Effect of steam sterilization on the water-soluble matter in soil. N. Y. (Cornell) Agr. Exp. Sta. Bul. 275, p. 129–155.

(63) LYON, T. L., AND BIZZELL, J. A. 1913 Water-soluble matter in soils sterilized and reinoculated. N. Y. (Cornell) Agr. Exp. Sta. Bul. 326, p. 205–224.

(64) MANN, H. H. 1908 Report of the agricultural chemist. Ann. Rpt. Dept. Agr. Bombay, 1908–9, p. 50–54. *Abs. in* Exp. Sta. Rec., v. 23, p. 129.

(65) MARBUT, C. F., BENNETT, H. H., LAPHAM, J. E., AND LAPHAM, M. H. 1913 Soils of the United States. U. S. Dept. Agr. Bur. Soils Bul. 96.

(66) MCGEORGE, W. T. 1915 The effect of partial sterilization on plant growth. Hawaii Agr. Exp. Sta. Ann. Rpt. 1915, p. 37–38.

(67) MELLOR, J. W. 1916 Higher Mathematics for Students of Chemistry, and Physics, p. 521–524 and 528. Longmans, New York.

(68) MOSIER, J. G., AND GUSTAFSON, A. F. 1917 Soil Physics and Management, p. 178. Lippincott, Phila.

(69) NAGOAKA, 1911 Tokio Col. of Agr. Bul. 4, p. 265. *Ref. in* Wis. Agr. Exp. Sta. Res. Bul. 19, p. 5.

(70) NOYES, H. A. 1919 The effect of heat on the lime requirement of soils. *In* Jour. Amer. Soc. Agron, v. 11, p. 70–71.

(71) NOYES, H. A. 1919 Accurate determination of soil nitrates by phenoldisulphonic method. *In* Jour. Indus. Engin. Chem., v. 11, p. 213–218.

(72) PETERSON, P. P. 1911 Effect of heat and oxidation on the phosphorus of the soil. Wis. Agr. Exp. Sta. Res. Bul. 19, p. 1–16.

(73) PFIEFFER, TH., AND FRANKE, E. 1896 Beitrag zur Frage der Verwertung elementaren Stickstoffs durch den Senf. *In* Landw. Vers. Stat., v. 46, p. 117–151.

(74) PICKERING, S. U. 1908 Studies on germination and plant growth. *In* Jour. Agr. Sci., v. 2, p. 411–434.

(75) PICKERING, S. U. 1908 The action of heat and antiseptics on soils. *In* Jour. Agr. Sci., v. 3, p. 32–54.

(76) PICKERING, S. U. 1910 Studies of the changes occurring in heated soils. *In* Jour. Agr. Sci., v. 3, p. 258–276.

(77) PICKERING, S. U. 1910 Plant growth in heated soils. *In* Jour. Agr. Sci., v. 3, p. 277–284.

(78) POTTER, R. S., AND SNYDER, R. S. 1915 The determination of nitrates in soil. *In* Jour. Indus. Engin. Chem., v. 7, p. 863–864.

(79) POTTER, R. S., AND SNYDER, R. S. 1916 Extraction of nitrates from soil. *In* Jour. Amer. Soc. Agron., v. 8, p. 54–55.

(80) POTTER, R. S., AND SNYDER, R. S. 1918 The effect of heat on some nitrogenous constituents of soil. *In* Soil Sci., v. 5, p. 197–212.

(81) RAHN, OTTO 1907 Bakteriologische untersuchungen über das Trochnen des Bodens. *In* Centbl. Bakt. (etc.), Abt. 2, v. 20, p. 38–61.

(82) RICHTER, L. 1896 Ueber die Veränderung welcher Boden durch das sterilizieren erleidet. *In* Landw. Vers. Stat., v. 47, p. 269–274.

(83) RITTER, G. A. 1912 Das Trochnen der Erden. *In* Centbl. Bakt. (etc.), Abt. 2, v. 33, p. 116–143.

(84) ROBINSON, R. H. 1920 Concerning the effect of heat on the reaction between lime-water and acid soils. *In* Soil Sci., v. 9, p. 151–157.

(85) RUSSELL, E. J. 1910 The fertilizing effect of sunlight. *In* Nature (London), v. 83, p. 6, 249 and 490.

(86) RUSSELL, E. J., AND HUTCHINSON, H. B. 1909 The effect of partial sterilization of the soil on the production of plant-food. *In* Jour. Agr. Sci., v. 3, p. 111–144.

(87) RUSSELL, E. J., AND PETHERBRIDGE, F. R. 1913 On the growth of plants in partially sterilized soils. *In* Jour. Agr. Sci., v. 5, p. 248–287.

(88) SCHMOEGER, M. 1893 Ueber den Phosphor im Moorboden. *In* Ber. Deut. Chem. Gesel., Jahrgang 26, v. 1, p. 386–394.

(89) SCHREINER, OSWALD, AND FAILYER, G. H. 1906 Colorimetric, turbidity and titration methods used in soil investigations. U. S. Dept. Agr. Bur. Soils Bul. 31, p. 39–41.

(90) SCHREINER, OSWALD, AND LATHROP, E. C. 1912 The chemistry of steam heated soils. U. S. Dept. Agr. Bur. Soils Bul. 89, p. 1–37. *Also in* Jour. Amer. Chem. Soc., v. 34, p. 1142–1259.

(91) SCHULZE, C. 1906 Einige Beobachtungen über die Einwirkung der Bodensterilisation auf die Entwickelung der Pflanzen. *In* Landw. Vers. Stat., v. 65, p. 137–147.

(92) SEAVER, J. F., AND CLARK, E. D. 1910 Changes brought about by heating of soils and relation to the growth of pyronema and other fungi. *In* Mycologia, v. 2, p. 109–124.

(93) SEAVER, F. J., AND CLARK, E. D. 1912 Biochemical studies on soils subjected to dry heat. *In* Biochem. Bul. v. 1, p. 413–427.

(94) SKALSKIJ, S. 1912 Work of the chemical laboratory of the Ploti Experiment Station, 1912. *In* Godichnyi Otchet Ploti Selsk. Khoz. Opytn Stanstii, v. 18, p. 133–227. *Abs. in* Exp. Sta. Rec., v. 30, p. 216, 349–380.

(95) SKALSKIJ, S. 1916 Method of sterilizing and chloroforming soil used in studying chernozem. *In* Iuzh. Russ. Selsk. Khoz. Gaz., 1916, no. 1, p. 7–8; no. 2, p. 6–7; no. 5, p. 5–10. *Abs. in* Exp. Sta. Rec., v. 38, 17–18.

(96) STEWART, G. R. 1918 The effect of season and crop growth on soil extract. *In* Jour. Agr. Res., v. 12, p. 311–368.

(97) STONE, G. E., LODGE, O. A., AND SMITH, R. G. 1912 Influence of soil decoctions from sterilized and unsterilized soils upon bacterial growths. Mass. Agr. Exp. Sta. 24th Ann. Rpt., p. 126–134.

(98) STONE, G. E., AND MONOHAN, N. F. 1906 Report of the Botanist. Mass. (Hatch) Agr. Exp. Sta. 18th Ann. Rpt., p. 125–134.

(99) STONE, G. E., AND SMITH, R. G. 1898 Nematode worms. Mass. (Hatch) Agr. Exp. Sta. Bul. 55, p. 1–67.

(100) TACKE, B. 1897 Die Arbeiten im Laboratorium der Station in Bremen und die Feld, und Wiesenversuche in den bremischen Mooren. *In* Centbl. Agr. Chem., v, 26, p. 366–382.

(101) TACKE, BR., AND IMMENDORF, H. 1898 Untersuchungen über die Phosphorverbindungen des Moorbodens. *In* Landw. Jahrb., Suppl., v. 274, p. 303–348.

(102) WARINGTON, R. 1882 On the determination of nitric acid in soils. *In* Jour. Chem. Soc. (London), Trans., v. 41, p. 351–360.

(103) WHITNEY, M., AND CAMERON, F. K. 1903 The chemistry of the soil as related to crop production. U. S. Dept. Agr. Bur. Soils Bul. 22, p. 1–71.

(104) WHITSON, A. R. 1903 Studies in the development and distribution of nitrates and total water-soluble salts in field soils. Wis. Agr. Exp. Sta. 20th Ann. Rpt., p. 339–344.

(105) WILSON, A. 1915 Changes in soil brought about by heating. *In* Sci. Proc. Roy. Dublin Soc., v. 14 (n. s., no. 38), p. 513–520.

(106) WILSON, G. W. 1914 Studies of plant growth in heated soils. *In* Biochem. Bul., v, 3, p. 202–209.

SULFUR OXIDATION IN "BLACK ALKALI" SOILS[1]

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In 1916, Lipman (6)[2] made the suggestion that the change of sulfur to sulfuric acid in the soil caused by the action of microörganisms might be employed for converting sodium carbonate into sodium sulfate and making productive the barren alkali areas. Lipman and Sharp (5) showed in their studies of chemical treatment of alkali land that where about 50 tons of strong sulfuric acid were sprayed on 40 acres of alkali soil there was a decided improvement in the productive power of the soil, but not great enough to justify the sulfuric acid treatment in practice. Treatment with sulfur gave no results.

For the study of the behavior of inoculated sulfur in black alkali soils, two 200-pound samples of such soil were secured by courtesy of Professor Hoagland of the University of California. For information concerning one of the alkali soils the following data were given by Dr. Hoagland:

> This soil was obtained from the University Ranch near Fresno in the San Joaquin Valley, California. It came from a bad spot in a tract of 160 acres called the "experimental drainage tract." This land was originally productive but later ceased to produce crops on account of the accumulation of the so-called alkali salts. Some years ago an attempt was made to reclaim this soil by installing a tile drainage system five or six feet deep and by flooding twice with an excess of good irrigation water. An attempt was then made to grow a barley crop but it was found that the reclamation was not successful as less than one-half of the land was capable of even fair production. Many spots were bare. . . . . Our present information leads us to believe that it is almost impossible to remove the toxic alkalinity from this soil by ordinary washing. It undoubtedly contains various alkaline substances including sodium carbonate, sodium silicate, sodium organic compounds, etc. The physical state of the soil is exceedingly bad.

The given information is undoubtedly about soil no. 17 for, although this soil will not support any ordinary growth as has been repeatedly tried with different crops in the greenhouse, and although it has a high alkalinity and a high content of alkali salts, the alkalinity and the amounts of salts are not as high as in soil no. 16. Moreover, soil no. 17 has a decidedly lower chlorine content than soil no. 16. Both the soils are sandy loam soils with a very

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[2] In the course of a study on sulfur oxidation Dr. J. G. Lipman suggested the work reported in this paper.

fine structure. This can be seen from the data given in table 1, showing the results of the mechanical analyses of these soils.

The poor structure of this soil as well as the high alkalinity prevents ordinary crops from taking hold, so in addition to changing the alkalinity, the structure ought to be improved simultaneously.

Chemical analyses of these soils were made only so far as seemed necessary for the scope of the study. K_2O, P_2O_5, Cl, N, SO_4, Ca and total alkalinity were determined. Alkalinity was determined by titration and hydrogen-ion concentration methods.

TABLE 1

Mechanical analyses of soils number 16 and 17

NAME OF FRACTION	NO. 16	NO. 17
	per cent	*per cent*
Fine gravel	4.94	5.21
Coarse sand	11.14	12.07
Fine sand	7.85	9.56
Very fine sand	28.10	31.52
Silt and clay	47.56	41.42

INFLUENCE OF SULFUR OXIDATION ON THE ALKALINITY OF THE SOILS

To these soils was added inoculated sulfur in amounts varying from 100 to 3500 pounds per acre, and the highest amounts of sulfur added were calculated to be adequate for the neutralization of the high alkalinity of soil no. 16. The different applications were made for the purpose of determining whether or not sulfur oxidation would occur in smaller quantities in these soils evidently toxic to the biological flora and to plant growth. Although the same quantities of sulfur were applied to soil no. 17, the greater amounts were more than necessary to neutralize the alkalinity of the soil.

The soil samples were thoroughly mixed with the inoculated sulfur, and were then placed in tumblers covered with glass plates. The tumblers with contents were weighed and the water-holding capacity of the soil determined. The soils were kept at 60 per cent of their water-holding capacity throughout the experiment and incubated at 28°C.

During the incubation period a record was kept of the changes in hydrogen-ion concentrations, which for soil no. 16 are recorded in table 2.

It can be seen at once that the smallest sulfur applications exerted little or no influence on the change of the hydrogen-ion concentration, but the higher amounts of inoculated sulfur brought about a decided lowering of the hydrogen-ion concentration. However, after an incubation period of 30 weeks, the neutral point was not yet reached.

Much more pronounced were the changes of the hydrogen-ion concentration caused by sulfur oxidation in soil no. 17, which changes are reported in table 3.

The changes in hydrogen-ion concentration occurring during the relatively short incubation period of 12 weeks were such that in several cultures the neutral point was reached before, or at the end of this period. This indicated that if sufficient sulfur is added, the sulfur changing to sulfuric acid could be

TABLE 2

Changes in hydrogen-ion concentrations of water extracts from soil no. 16 during the period of incubation

CULTURE NO.	SULFUR APPLIED	INITIAL pH VALUE	pH VALUES AFTER VARYING PERIODS OF INCUBATION									
			2 weeks	3 weeks	5 weeks	6 weeks	8 weeks	9 weeks	11 weeks	12 weeks	18 weeks	30 weeks
	lbs. per acre											
12	None	9.8	9.7	9.7	9.8	9.9	9.9	9.9	9.7	9.6	9.6	9.6
13	100	9.8	9.7	9.6	9.5	9.8	9.8	9.8	9.6	9.7	9.6	9.6
14	300	9.8	9.4	9.5	9.5	9.7	9.8	9.8	9.6	9.6	9.6	9.6
15	500	9.8	9.4	9.6	9.6	9.8	9.8	9.7	9.7	9.6	9.5	9.5
16	1000	9.8	9.5	9.5	9.6	9.8	9.7	9.8	9.7	9.3	9.3	9.4
17	1500	9.8	9.8	9.7	9.6	9.6	9.6	9.6	9.5	9.3	9.3	9.3
18	2000	9.8	9.7	9.5	9.4	9.3	9.3	9.3	9.4	9.2	9.2	9.2
19	2250	9.8	9.7	9.4	9.2	9.3	9.3	9.3	9.4	9.1	8.8	8.7
20	2500	9.8	9.8	9.4	9.3	9.3	9.3	9.2	9.1	9.0	8.6	8.7
21	3000	9.8	9.6	9.4	9.3	9.2	9.2	9.3	9.1	8.9	8.2	8.2
22	3500	9.8	9.8	9.8	9.8	9.7	9.5	9.3	8.7	8.2	8.0	8.0

TABLE 3

Changes in hydrogen-ion concentrations of water extracts from soil no. 17 during the period of incubation

CULTURE NO.	SULFUR APPLIED	INITIAL pH VALUE	pH VALUES AFTER VARYING PERIODS OF INCUBATION									
			2 weeks	3 weeks	5 weeks	6 weeks	8 weeks	9 weeks	11 weeks	12 weeks	18 weeks	30 weeks
	lbs. per acre											
23	None	9.2	9.1	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2
24	100	9.2	9.2	9.2	9.2	9.0	9.0	9.0	8.9	8.8	8.8	8.8
25	300	9.2	9.2	9.1	9.0	9.0	9.0	9.0	8.8	8.6	8.6	8.6
26	500	9.2	9.0	8.7	8.9	9.0	8.9	9.0	8.6	8.4	8.4	8.4
27	1000	9.2	9.0	8.6	8.3	8.6	8.5	8.5	8.2	8.0	8.0	8.1
28	1500	9.2	8.8	8.1	7.8	7.7	7.4	7.4	7.4	7.4	7.6	7.8
29	2000	9.1	7.7	7.8	8.0	8.1	8.1	8.0	7.9	7.4	7.4	7.6
30	2250	9.0	7.6	7.5	7.4	7.3	7.3	7.3	7.2	7.0	6.9	7.3
31	2500	8.9	7.5	7.4	7.6	7.5	7.4	7.3	7.3	7.0	6.9	7.2
32	3000	8.9	7.4	7.4	7.4	7.3	7.2	7.1	7.0	6.8	6.6	7.0
33	3500	9.0	7.5	7.4	7.3	7.2	7.0	6.9	6.3	6.6	6.4	6.7

employed to convert the sodium carbonate into sodium bicarbonate and sodium sulfate. The results from inoculated sulfur applications would be noticed within a reasonably short time, provided the soil had been subjected to leaching previous to the sulfur treatment. It is interesting to notice

that in both soils very little change in reaction took place after 12 weeks, not even in the cultures treated with the larger quantities of sulfur. In fact the process of oxidation seemed nearly completed after this period. It seems, therefore, that in neutralizing the soil a somewhat greater quantity of sulfur is necessary than the amount theoretically calculated.

These experiments were repeated with a second lot of two samples of about 100 pounds each, sent by courtesy of Dr. Hoagland from the same place. The soils were again labelled 16 and 17. For convenience they will be referred to as numbers 16a and 17a. These experiments were conducted in the greenhouse with glazed earthenware pots having a capacity of 2.5 kgm. The experiments were conducted during the months of November to April.

To the soils were added the following quantities of sulfur:

CULTURE NO.	POUNDS PER ACRE	CULTURE NO.	POUNDS PER ACRE
1 and 10	None	6,15	2000
2 and 11	300	7,16	2500
3 and 12	500	8,17	3000
4 and 13	1000	9,18	3500
5 and 14	1500		

The pots were left standing on a bench in the greenhouse and watered at intervals, but no care was taken to keep the soils at an optimum moisture content. Soon heavy salt incrustations were formed at the surface, but the soil was not stirred and no attempt was made to remove the crystalized salts other than by adding water. This may explain why slight irregularities occured in the readings of the hydrogen-ion concentrations. For sampling, a wide cork borer was used and care was taken to have a column of soil from top to bottom, without destroying the layer of salts on top of the soils.

The changes in hydrogen-ion concentration recorded are reported in table 4.

The changes of hydrogen-ion concentration in these soils placed in pots were very similar to the changes brought about by inoculated sulfur in the tumblers kept at a supposed optimum moisture content and a regulated temperature. Slight changes took place in the soils to which 300 and 500 pounds of sulfur to the acre were added until the end of the eight or the end of the tenth week, particularly in soil no. 17a. After this time the hydrogen-ion concentration became less, and returned toward the original concentration. As could be expected the higher application of sulfur exerted a far greater influence on the change of the hydrogen-ion concentration than the small amounts added. After 12 weeks the neutral point was reached in the case of soil no. 17a to which 3500 pounds of sulfur was added, and where smaller quantities of sulfur were applied the hydrogen-ion concentration approached the neutral point.

These soils do not support any ordinary plant growth. An attempt was made to determine at what hydrogen-ion concentration barley seedlings would survive and start to grow. Every 14 days barley seedlings were planted in all pots, during the first 10 weeks without being able to keep the seedlings alive. In the soils to which large quantities of sulfur were added the plantlets made considerable growth but because of the small capacity of the pots and the imperviousness of the soil the plants were injured by the salt solution concentrating at the surface of the soil. The pots were watered frequently and the water added dissolved the hard alkali incrustations formed on

TABLE 4

Changes in hydrogen-ion concentrations of soils no. 16a and 17a treated with inoculated sulfur and placed in the greenhouse

SOIL NO.	CULTURE NO.	SULFUR APPLIED	INITIAL REACTION	pH VALUES AFTER VARYING PERIODS OF INCUBATION										
				1 week	3 weeks	5 weeks	6 weeks	7 weeks	8 weeks	10 weeks	11 weeks	12 weeks	18 weeks	24 weeks
		lbs. per acre												
	1	None	9.6	9.6	9.6	9.6		9.6		9.6	9.6	9.6	9.6	9.6
	2	300	9.6	9.4	9.4	9.4		9.6		9.0	9.1	9.2	9.0	9.1
	3	500	9.6	9.4	8.9	9.0		8.9		8.8	8.9	9.1	8.9	8.9
	4	1000	9.6	9.4	9.4	8.9		9.0		8.4	8.5	8.6	8.3	8.4
16a	5	1500	9.6	9.4	8.9	8.7		8.4		8.2	7.9	7.6	8.0	7.7
	6	2000	9.5	9.4	8.8	8.1		8.0		8.0	7.9	7.9	8.1	7.7
	7	2500	9.6	9.4	8.6	8.1		8.1		7.7	7.6	7.6	8.0	7.6
	8	3000	9.6	9.5	8.5	7.9		8.1		7.5	7.6	7.7	8.0	7.5
	9	3500	9.6	9.5	8.2	7.7		8.0		7.6	7.6	7.5	7.3	7.1
	10	None	9.2	9.2	9.0	9.2	9.1	9.2	9.0		9.0	9.2	9.2	9.3
	11	300	9.2	9.2	9.2	9.1	9.0	8.8	8.6		9.0	9.2	9.0	9.1
	12	500	9.1	9.2	9.0	8.9	9.0	8.6	8.6		9.0	9.1	9.1	9.1
	13	1000	9.2	9.1	8.9	8.6	8.8	8.4	8.4		8.8	8.6	8.7	8.6
17a	14	1500	9.2	9.1	9.1	8.7	8.6	8.0	8.0		7.7	7.9	8.4	8.4
	15	2000	9.2	9.1	9.0	8.5	8.6	8.0	8.0		8.0	7.9	8.3	7.6
	16	2500	9.1	9.2	9.0	8.4	8.4	7.7	7.5		7.4	7.5	7.6	7.5
	17	3000	9.1	9.2	8.8	8.2	8.2	7.9	7.5		7.3	7.4	7.7	7.5
	18	3500	9.1	9.1	8.7	8.2	8.1	7.5	7.3		6.6	6.8	7.0	6.8

top of the soil. Because of the poor physical condition of the soil this solution remained on the surface a considerable length of time and finally killed the plantlets.

Barley seeds were then planted, and they germinated well in soil no. 17a receiving 1500 pounds or more of sulfur per acre. Only a few seeds germinated in soil no. 16a to which 2500 or more pounds of sulfur per acre was applied. All these seedlings grew well, had a very dark green color, but most of them were not able to survive the injury caused by the repeated flooding and consequent attack of the concentrated salt solution. It seems reasonable to

expect far better results in the field, or in pots with a device to take care of the watering or at least of the surplus of water. These soils have an extremely poor structure and would be difficult to handle even without injurious "alkali" salts.

INFLUENCE OF SULFUR OXIDATION ON THE BIOLOGICAL FLORA

For the study of the biological changes, infusions were made from soils no. 16 and 17, incubated at 28°C., and plated out on Lipman and Brown's agar at the beginning of the experiment, after 6 weeks, after 12 weeks, and after 18 weeks. The biological flora for these soils expressed in numbers of colonies growing on agar plates as an average of three plates counted is given in table 5.

TABLE 5

Bacterial numbers in soil no. 16 and 17 treated with inoculated sulfur (in thousands per gram)

SULFUR APPLIED, (*lbs. per acre*)	NONE	100	300	500	1000	1500	2000	2250	2500	3000	3500
Culture No.	1	2	3	4	5	6	7	8	9	10	11
Soil no. 16											
Before treatment	60	58	64	62	63	60	59	60	58	62	60
After 6 weeks	61.5	30	35	65	50	60	181	231	230.5	226	255
After 12 weeks	69	75	105.5	125.5	107	112.5	208	251	302	348	411
After 18 weeks	70	74	102	116	122	158	241	306	388	492	627
Soil no. 17											
Before treatment	335	332	333	334	335	332	318	329	326	334	329
After 6 weeks	330	325	320	275	260	250	225	255	250	235	255
After 12 weeks	435	442	490	540	630	960	1700	1225	1550	1600	2100
After 18 weeks	440	448	520	620	752	1110	1720	1542	1832	2540	2940

It seems evident that the numbers of colonies growing on agar plates varied directly as the hydrogen-ion concentration. The rate of increase was very much the same for both soils.

The increase in colonies was quite consistent with the increase of the amount of sulfur added. Still, the highest numbers obtained were relatively small if compared with the numbers present in ordinary soils. The numbers in the untreated incubated soils seemed to increase slightly, as could be expected from the favorable temperature and moisture content. After an incubation period of 12 weeks, the soil receiving 3500 pounds of sulfur per acre, was found to produce approximately five times as many colonies per plate as the untreated soil. The numbers for the cultures with the higher sulfur applications for soil no. 17 were from three to five times greater.

It was noted throughout all the experiments in the three different soils to which certain quantities of sulfur were added that a relatively large number

of tiny white colonies of actinomycetes appeared on the surface of the soil. In fact, if the soils were very acid or extremely alkaline, the counts made on the agar plates usually consisted for the most part of molds and actinomycetes. The molds predominated in the cultures to which very small amounts of sulfur were added and the actinomycetes in the cultures with greater quantities of sulfur. Often two-thirds or even four-fifths of the colonies on the plates inoculated with infusions from the soil with the lower sulfur applications, were molds and actinomycetes. In the alkaline soil no. 17 to which larger amounts of sulfur were added and which reached or approached the neutral point but a very small number of the total colonies counted consisted of molds and actinomycetes. It was surprising to notice the difference which occured. Nevertheless, not a great variety of biological flora was present.

INFLUENCE OF SULFUR OXIDATION ON THE PHYSICAL STRUCTURE OF THE SOILS

The influence of chemicals, salts and various substances upon soils has been studied in innumerable instances. There is no doubt about the important part played by the various constituents added to a soil. The work reported in this part of the paper was carried out mainly to study the change in the physical structure of the soils when inoculated sulfur was added, and transformed into sulfates. In the course of the biological and vegetation experiments it was frequently noticed that the soil, to which different amounts of sulfur were added, had a tendency to form small aggregates, resulting in making the soils apparently more porous.

Unfortunately no good methods are available for measuring accurately the changes in granulation. The methods employed to estimate small differences caused by additions of salt could not be used on account of obvious obstacles. An attempt was made to determine differences in granulation, produced as a result of sulfur oxidation, by screening the soils, but slight differences in the moisture contents of the soils produced greater errors than the differences found between the soils treated with varying amounts of inoculated sulfur. Drying the soils in the oven made no difference in the size of the errors.

An effort made to measure the differences in cohesion of the soils as described by Cameron and Gallagher (1), and again by Davis (2), by means of an apparatus for measuring penetration, failed likewise to gave reliable results.

Patten and Waggaman (7) have shown that adsorption of soluble material exerts an appreciable influence upon the water-holding power of finely divided soils. The hydroxides and carbonates seemed to lower the water-holding capacity and other salts to raise it. An attempt was made to determine the influence of the formation of sulfates in the soils, upon the water-holding capacity.

The endeavor to measure the formation of aggregates, by measuring the capillary rise of water, failed because the soils had to be packed closely thereby destroying the small aggregates formed.

The measured changes from carbonates into bicarbonates, flocculation and turbidity measurements can hardly bring out the phenomonon of aggregate formation. Determinations of apparent specific gravity of the treated and untreated soils also are apt to produce errors, even if the samples are previously dried, since slight differences in the initial percentage of water may cause irregularities.

Nevertheless, all these interdependent factors together can give some idea of the changes caused by the oxidation of elemental sulfur into sulfates by microörganisms.

Methods

Water-holding capacity determinations of the treated and untreated soil were made according to the Hilgard (3) method after 12 weeks of incubation. Averages of three determinations are given in the tables.

The method used to determine the change in apparent specific gravity of the soils was that of weighing, full of soil, a vessel whose volume was known. The dried soil was allowed to fall through a coarse wire creen until it overran the edges of the cup, and was then carefully leveled to the top of the cup with the edge of a spatula as recommended by Davis (2). After weighing the cup and soil, the top of the soil was taken off and a representative sample of the interior of the mass placed in a weighing bottle and the moisture determined by heating for 15 hours at 105 to 108°C. Triplicate moisture determinations showed that but little difference occurred between the previously dried samples, and an average of these three determinations was taken as a basis for calculations.

Determinations of apparent specific gravity on samples not previously dried showed greater differences but were discarded. At least two determinations were made of all samples.

Flocculation of the soil was determined by shaking 2 gm. of soil with 50 cc. of distilled water using burettes as containers, and measuring as accurately as possible the amount of the clear or transparent liquid, after standing for 24 hours.

For the turbidity measurements, one part of soil was mixed with two parts of water, the soil suspensions were poured into burettes, and after 24 hours the upper 10 cc. was carefully pipetted off into weighing dishes and the total residue estimated after evaporation and drying at 100°C. as has been suggested by Hoagland and Martin (4).

Total alkalinity and bicarbonates were determined by making water extractions of 20 gm. of air-dried samples of each soil. The samples were shaken with 200 cc. of distilled water in a shaking machine for 2 hours. The flasks were left standing over night and filtered until the liquid was clear or transparant without sediment. An aliquot was titrated with 0.02*N* sulfuric acid with methyl red and phenolphtalein as indicators.

In the cases of the alkali soils a few cubic centimeters of concentrated hydrochloric acid were added to the remainder of the liquid to aid settlement of possible small quantities of colloidal matter.

Soluble sulfate formed was determined in aliquots of the liquid to which was added 2 cc. of concentrated hydrochloric acid, by precipitating at the boiling point with barium chloride.

Experimental results

A. Soil No. 1

In order to be able to compare the results obtained from the alkali soils with a productive soil there was included a series of cultures of a good Hanford sandy loam. This soil was taken from an orange grove, which had been manured annually with barnyard manure. It was slightly alkaline by the hydrogen-ion method, having a pH value of 7.2. It was neutral with the Veitch method, but after shaking for several hours in the shaking machine the liquid had a total titratable alkalinity of 0.21 cc. of 0.1 *N* H_2SO_4 per 10 gm. of soil.

The formation of sulfate in soils treated with inoculated sulfur is influenced by the water content of the soil. Experiments were carried out to determine at which point of water saturation sulfur oxidation would reach the optimum for the soils used when at a temperature of 28°C. It was found that oxidation increased with the increase of moisture until approximately 55 per cent of the amount necessary for complete saturation was reached. From then on oxidation decreased slowly to about 70 per cent of the water-holding capacity. When larger quantities of water were added hydrogen sulfide began to develop, which could easily be detected by the smell and sometimes even by the black color produced in the mixtures.

It was found in earlier experiments that sulfur oxidation proceeded more rapidly at a temperature of 30°C. than at room temperature. The cultures under discussion were kept at 28°C. throughout the experiments. This temperature, however, does not necessarily mean best for most rapid sulfur oxidation.

The data given in table 6 show that with the increase of the amount of inoculated sulfur an increase occured in water-soluble sulfates. The increase obtained, however, was not proportional to the increase of sulfur added, as is reported in another table.

With the increase of sulfate accumulation the titratable acidity naturally increased.

The soil to which inoculated sulfur was added seems to assume a new set of physical properties, characterized by a more or less complete flocculation. The increase of sulfate accumulation was nearly proportional to the increase in flocculation, which is very rapid when greater quantities of sulfur are employed. From the data presented it is evident that there exists a good cor-

relation between the quantities of suspended material as measured by the transparent liquid column, the quantities of this material weighed after drying (turbidity), and the amounts of sulfur oxidized.

A number of investigators have set forth the influence of various hydroxides, carbonates, phosphates, sulfates, etc. upon the water-holding power of soils. Although the results obtained are irregular, there seems to be a general tendency to decrease the water-holding capacity with the increase of sulfates formed.

The data obtained and presented in table 6 for the changes in apparent specific gravity seem to indicate that the formation of sulfates caused the soil to increase in volume up to a certain point. After this point was reached the volume decreased again. It should be born in mind, however, that not

TABLE 6

Formation of sulfates, changes in total acidity, flocculation, turbidity, water-holding capacity and apparent specific gravity in soil no. 1, after 12 weeks of incubation

CULTURE NO.	SULFUR APPLIED	SULFATE*	TOTAL ACIDITY IN TERMS 0.1 *N* NaOH*	FLOCCULATION MEASURED BY TRANSPARENT LIQUID	TURBIDITY MEASURED BY RESIDUE IN SOIL SUSPENSION	WATER-HOLDING CAPACITY	SPECIFIC GRAVITY
	lbs. per acre	*mgm.*	*cc.*	*cc.*	*mgm.*	*per cent*	
1	None	1.07	0.02	7.5	24.2	31.9	126.83
2	100	2.39	0.05	8.0	23.9	32.7	126.02
3	300	5.38	0.20	8.0	22.9	31.3	126.22
4	500	7.15	0.28	11.5	18.4	31.2	124.32
5	1000	15.68	0.30	12.0	13.2	31.4	123.12
6	1500	17.92	0.32	12.2	6.2	33.1	118.17
7	2000	20.52	0.40	14.1	4.7	31.4	113.08
8	2250	19.08	0.40	16.3	2.0	27.5	113.08
9	2500	24.20	0.50	24.2	2.2	28.7	114.81
10	3000	24.86	0.60	32.4	1.9	29.0	116.08
11	3500	27.80	0.64	50.0	1.8	28.6	118.27

* Per 10 gm. of soil.

all the sulfur added had been oxidized, and the quantities of oxidized sulfur necessary to bring about greatest increase in volume may be different from the quantities applied where this point was observed and as is indicated by the figures in the table.

B. Soil No. 16

The oxidation of sulfur as indicated by the water-soluble sulfates increased with the increase of the quantities of inoculated sulfur employed as shown in table 7.

It is very evident that the carbonates were transformed into bicarbonates with the decrease in alkalinity. It is obvious that the toxic carbonates were changed to a great extent. In fact, almost all titrated alkalinity in the soil to which the largest amount of sulfur was added, had been converted into

bicarbonates, although the neutral point, with phenolphtalein as indicator, was by no means reached at the end of an incubation period of 12 weeks, and high alkalinity and large amounts of salts still prevailed. The soil extract was nearly neutral to methyl red.

With the decrease of alkalinity an increase in flocculation occurred, but this increase was, if compared with soil no. 1, not very large. The figures showing turbidity measurements include the salts present after shaking with distilled water for 2 hours in a shaking machine, and therefore, do not represent the true turbidity, since the drying of the residue does not remove the salts present. It was thought better not to try to deduct the amounts of

TABLE 7

Formation of sulfates and bicarbonates, changes in total alkalinity, flocculation, turbidity, water-holding capacity and apparent specific gravity in soil no. 16, after 12 weeks of incubation*

CULTURE NO.	SULFUR APPLIED	SULFATE†	BICARBONATES IN TERMS OF 0.02 *N* H_2SO_4	TOTAL ALKALINITY IN TERMS OF 0.1 *N* H_2SO_4†	FLOCCULATION MEASURED BY TRANSPARENT LIQUID	TURBIDITY (SALTS INCLUDED) MEASURED BY RESIDUE IN SOIL SUSPENSION	WATER-HOLDING CAPACITY	SPECIFIC GRAVITY
	lbs. per acre	*mgm.*	*cc.*	*cc.*	*cc.*	*mgm.*	*per cent*	
12	None	9.71	1.04	4.68	1.0	231.2	28.2	123.72
13	100	13.98	1.00	4.60	1.5	231.4	28.2	123.02
14	300	14.26	1.98	4.61	1.7	231.0	27.3	122.30
15	500	14.88	1.82	4.58	4.0	230.4	27.7	121.98
16	1000	15.72	1.72	4.52	4.0	230.0	26.6	121.55
17	1500	16.76	2.04	4.52	6.1	229.6	28.2	120.44
18	2000	26.32	2.54	4.47	5.2	229.0	28.9	118.88
19	2250	35.79	2.86	4.47	6.2	227.0	30.0	120.38
20	2500	37.38	2.79	4.32	7.1	227.1	28.0	115.96
21	3000	39.02	2.78	4.20	7.3	221.5	27.9	117.11
22	3500	42.38	3.20	3.60	9.2	221.0	30.7	115.38

* After the work was completed a short paper appeared in Soil Science, v. 9, p. 385, by P. L. Hibbard, showing the effect of sulfur oxidation on the reduction of alkalinity in alkali soils. Hibbard's titration data agree in general with the figures given in this table.

† Per 10 gm. of soil.

salts present by calculation, since the quantities of salts in the liquid after shaking would be necessarily different for the different cultures on account of the sulfur oxidized. Still, an appreciable decrease in turbidity occured, the weighed amounts gradually decreasing with the increase of the quantities of sulfur added.

The apparent specific gravity decreased with the increase of sulfate formation, being 9.3 per cent less for culture no. 22 as compared with culture no. 12.

C. Soil No. 17

Although sulfur oxidation did not seem to be greater in soil no. 17 than in soil no. 16, a number of cultures were neutral toward phenolphtalein at the

end of the incubation period. The former soil, having been leached, is considerably less alkaline and this accounts for the fact that much less inoculated sulfur was necessary to produce neutrality or even acidity after such a comparatively short incubation period. The remaining alkalinity in cultures no. 29 to 31 after 12 weeks was due to bicarbonates, and with the advance of sulfur oxidation the cultures gradually become neutral to phenolphtalein.

The turbidity measurements indicate that the physical structure of the soil was changed materially through the addition of inoculated sulfur.

The water-holding power of this soil seemed also to be changed, and the apparent specific gravity was decreased to a considerable extent, showing that there had been a formation of aggregates as a result of the sulfur oxidation. The changes under discussion are recorded in table 8.

TABLE 8

Formation of sulfates and bicarbonates, changes in total alkalinity, flocculation, turbidity, water-holding capacity and apparent specific gravity in soil no. 17, after 12 weeks of incubation*

CULTURE NO.	SULFUR APPLIED	SULFATE†	BICARBONATES IN TERMS OF 0.02 *N* H_2SO_4	TOTAL ALKALINITY IN TERMS OF 0.1 *N* H_2SO_4†	FLOCCULATION MEASURED BY TRANSPARENT LIQUID	TURBIDITY (SALTS INCLUDED) MEASURED BY RESIDUE IN SOIL SUSPENSION	WATER-HOLDING CAPACITY	SPECIFIC GRAVITY
	lbs. per acre	*mgm.*	*cc.*	*cc.*	*cc.*	*mgm.*	*per cent*	
23	None	7.65	1.00	3.52	0.5	98.0	27.2	124.62
24	100	9.11	1.76	3.02	1.5	94.1	27.4	123.92
25	300	13.42	0.72	2.40	3.0	87.2	27.1	121.40
26	500	16.10	0.92	1.80	1.5	79.0	29.2	121.20
27	1000	17.19	1.02	1.50	2.5	71.3	27.8	118.70
28	1500	20.27	1.02	1.20	5.0	65.1	28.5	118.33
29	2000	23.37	0.40	0.40	8.0	59.2	32.0	116.15
30	2250	34.59	0.40	0.40	16.2	54.0	33.3	116.34
31	2500	39.10	0.20	0.21	31.1	52.0	33.2	113.27
32	3000	42.06	0.00	0.00	47.2	43.2	34.9	114.61
33	3500	43.48	Acid	Acid	48.1	37.1	32.0	113.84

* See note at bottom of table 7.

† Per 10 gm. of soil.

The formation of sulfates may be directly responsible for the forming of aggregates but it is likely that the changes in the physical structure of all three soils under discussion is caused by the interrelated factors as the changes in microbiological flora, sulfate formation, conversion of carbonates into bicarbonates, and a possible interchange of bases in the soil.

The interdependence of the different phenomona recorded in the tables is brought out very strikingly in the graphs of figure 1. The curves representing sulfate formation are inverted to show the relation more clearly.

The exact quantities and percentages of inoculated sulfur oxidized, as far as they are changed into the water-soluble form, at the end of an incubation period of 12 weeks for the different cultures under discussion, are given in table 9.

In several instances sulfur oxidation in soils no. 16 and 17 had apparently been greater than in soil no. 1, especially in the cultures to which larger amounts of inoculated sulfur had been applied. It may be that in some instances not all soluble sulfates were removed by the shaking or that small

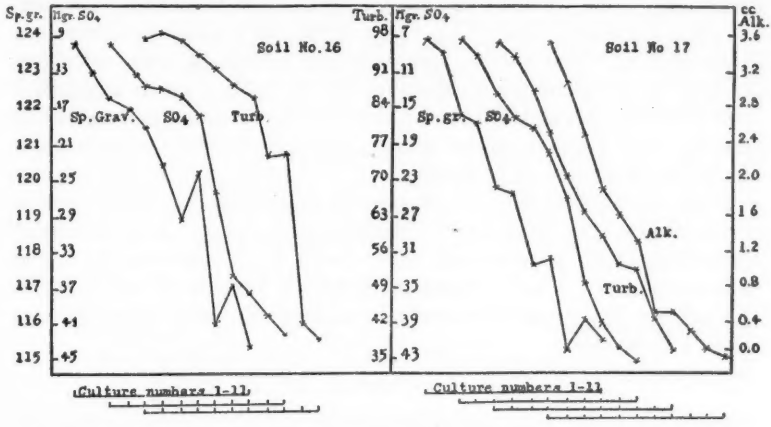

FIG. 1. CURVES REPRESENTING APPARENT SPECIFIC GRAVITY, SULFATES FORMED, TURBIDITY, AND CHANGES IN ALKALINITY OF SOILS NUMBERS 16 AND 17 AFTER AN INCUBATION PERIOD OF 12 WEEKS.

Abscissae represent culture numbers 1 to 11. To facilitate reading, the curves are placed next to each other instead of superimposing them.

TABLE 9

Quantities of inoculated sulfur oxidized in 100 gm. soil at the end of the incubation period of 12 weeks

CULTURE NO.	SULFUR APPLIED	SOIL NO. 1, SULFUR OXIDIZED		SOIL NO. 16, SULFUR OXIDIZED		SOIL NO. 17, SULFUR OXIDIZED	
	mgm.	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*
1	5	4.4	88.0	4.7	94.0	3.0	60.0
2	15	14.7	98.0	15.0	100.0	14.5	96.7
3	25	24.1	96.4	17.2	68.9	20.3	81.2
4	50	50.3	100.6	20.0	40.0	37.2	74.4
5	75	56.2	74.9	23.5	31.3	42.8	57.1
6	100	64.8	64.8	58.7	58.7	52.4	52.4
7	112	60.0	53.6	86.9	77.5	89.8	80.1
8	125	77.1	61.7	95.6	76.4	103.8	83.0
9	150	79.3	52.8	97.7	65.1	113.7	75.8
10	175	89.1	50.9	108.9	62.2	119.4	68.3

amounts of calcium sulfate, which are practically insoluble in distilled water, were formed. It can be seen from this table that but 50 to 60 per cent of the larger quantities of sulfur added had been oxidized in soil no. 1, whereas about 70 to 80 per cent of sulfur was oxidized in soil no. 17 to which the same quantities of sulfur were added.

Nearly all of the small amounts of sulfur added had been oxidized in all cultures. After 18 weeks the added sulfur had been oxidized in all cultures to an extent of 88 to 96 per cent, so that oxidation was practically completed at that time. It is obvious that in soil no. 17 a close relation exists between the readings of the pH values and the amounts of sulfur oxidized. The neutral point was reached after 81.5 per cent of the sulfur added to culture no. 8 was oxidized. This was approximately the amount of sulfur calculated necessary to neutralize the alkalinity of the soil. The cultures to which a surplus of sulfur was added and in which more than the amounts theoretically necessary were oxidized, showed acidity, although this acidity by the titration method hardly could be detected.

In soil no. 16 alkalinity still remained in all cultures, but the percentage of sulfur oxidized had also a great influence on the changes of the hydrogen-ion concentration. It seems that in all cases in these three soils the rate of oxidation was not entirely dependent upon the amounts of sulfur added, although the larger quantities naturally changed the high alkalinity more and at a faster rate.

SUMMARY

The soil to which inoculated sulfur is added seems to assume, through the formation of sulfates, a new set of physical properties characterized by a more complete flocculation, and changes in water-holding power and apparent specific gravity.

In "black alkali" soils small amounts of sulfur exerted little or no influence upon the hydrogen-ion concentration, but larger quantities brought about a decided change. The changes were similar in cultures kept in an incubator at 28°C. and at an optimum moisture-content, and in cultures kept in glazed earthenware pots in the greenhouse which were watered at indefinite intervals.

The leached soil was more readily neutralized by the addition of inoculated sulfur than the unleached soil, as indicated by titration and hydrogen-ion concentration methods. After 18 weeks the added sulfur had practically been oxidized in all cultures.

Carbonates were transformed into bicarbonates with the increase in sulfur oxidation. Although high alkalinity was changed into less alkalinity, high salt contents remained since no leaching occurred.

There was a striking relation between the sulfate formation and the flocculation, turbidity and apparent specific gravity of the leached and unleached soils. Sulfate formation tends to cause aggregation of this black alkali and Hanford sandy loam used, and the water-holding power seems to be changed.

The biological flora expressed in numbers of colonies grown on agar plates varied directly with the change in hydrogen-ion concentration. The cultures with the unleached soil, which received sufficient amounts of inoculated sulfur to neutralize the alkalinity, produced after 12 weeks of incubation, 5 times as many colonies per plate as did the untreated cultures, and the treated cul-

tures of the leached soil from 3 to 5 times as many colonies as the untreated cultures. After 18 weeks these cultures in both soils produced approximately 10 times as many colonies as did the untreated cultures.

The biological flora changed with the increase of the hydrogen-ion concentration. In the leached soil cultures, which reached or approached the neutral point after sulfur application, there were but relatively few colonies of molds and actinomycetes, whereas the colonies counted on agar plates made from infusions of untreated soil, consisted largely or nearly entirely of molds and actinomycetes.

Barley seeds germinated and grew readily in alkali soil treated with inoculated sulfur, but the plantlets were always killed by the salt incrustations on top of the soil, which dissolved when the pots were watered, while the water penetrated the soil but slowly on account of its poor physical condition.

REFERENCES

(1) CAMERON, F. K., AND GALLAGHER, F. E. 1908 Moisture content and physical condition of soils. U. S. Dept Agr. Bur. Soils Bul. 50.

(2) DAVIS, R. O. E. 1911 The effect of soluble salts on the physical properties of soils. U. S. Dept. Agr. Bur. Soils Bul. 82.

(3) HILGARD, E. W. 1906 Soils, Their Formation, Properties, Compositions and Relations to Climate and Plant Growth in Humid and Arid Regions. MacMillan, New York.

(4) HOAGLAND, D. R., AND MARTIN, J. C. 1920 Effect of season and crop growth on the physical state of the soil. *In* Jour. Agr. Res. v. 20, p. 397–404.

(5) LIPMAN, C. B., AND SHARP, L. T. 1919 Alkali investigations. *In* Cal. Agr. Exp. Sta. Rpt. 1918–1919, p. 65–67.

(6) LIPMAN, J. G. 1916 Sulfur on alkali soils. *In* Soil Sci., v. 2, p. 205.

(7) PATTEN, H. E., AND WAGGAMAN, W. H. 1908 Absorption by soils. U. S. Dept. Agr. Bur. Soils Bul. 52, p. 70.